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To test the hypothesis that estrogen enhances water and sodium retention, we compared the fluid regulatory responses to 150 min of exercise-induced dehydration, followed by 180 min of *ad libitum* drinking during the follicular and luteal phases of the menstrual cycle, and following treatment with an estrogen/progestin (OC E+P) and a progestin-only (OC P) oral contr ceptive in a double-blind, randomized, cross-over design. We found a downward shift in osmotic regulation of arginine vasopressin (AVP) during the luteal phase and OC E+P compared to the follicular phase during exercise, which was not accompanied by plasma volume expansion or fluid retention. Overall fluid and electrolyte balance was similar across all trials. We also determined that the variability of the fluid regulatory hormones (renin, aldosterone and atrial natriuretic peptide) was high over the course of two menstrual cycles, but slopes and intercepts defining osmotic regulation of AVP were highly reliable. We also determined the estrogen and progesterone on thermoregulation. We found that OC P increased core temperature and delayed the onset of sweating during exercise, but that OC E+ blocks these effects. This indicates that estrogen blocks the progestin-related changes in thermoregulation while on oral contraceptives. We also found that all of these effects were independent of changes in plasma volume. We have begun testing the impact of OC P and OC E+P on osmotic regulation of AVP during hypertonic saline infusion, but have completed only three subjects so have no comment at this point.

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Nina Stachenfeld Sept. 24 1999

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INTRODUCTION

Methods to regulate and suppress menstruation and provide contraception are needed as women take more active roles in the military. The administration of estrogen and progestin combinations in the form of the oral contraceptive pill has been proposed as a method to regulate menstruation in women during combat and field situations. Alternatively, some contraceptive pills provide progestins only, and contain no estrogen. Combined oral contraceptive pills contain synthetic estrogens, which exhibit 6-10 times the estrogenic activity provided by endogenous, circulating estrogens. Progestin-only pills not only contain no estrogen, but the unopposed progestin tends to down-regulate estrogen receptors. Thus, these two widely used oral contraceptive preparations differ significantly in their estrogenicity. Estrogens have potent effects on the regulation of body water balance (2, 8), so these two forms of oral contraceptive pills may differ in their effects on water regulation, and hence on physical performance under adverse environmental conditions.

Protocol A: Sex Hormone Effects on Body Water Regulation during Dehydration and Rehydration.

Sex hormone administration is accompanied by significant water and sodium retention (2, 8) which leads to plasma volume expansion (5, 7, 78, 87). In fact, variations in plasma volume at rest and during exercise that are observed following estrogen administration and during different phases of the menstrual cycle are comparable to the reported effects of posture, skin temperature and exercise intensity (33). Bilateral oophorectomy results in a 25% loss of blood volume, and replacement of estrogen restores blood volume (28). Oral contraceptive agents, which deliver pharmacological levels of estrogens, increase total body water (8). Fortney et al. (25) demonstrated an attenuation of the blood volume loss associated with bed rest following estrogen (premarin) administration. Some investigators have shown that plasma volume is higher during the follicular phase, when estrogen levels are rising (69, 70).

The mechanism underlying the estrogen-mediated body water retention is unclear, but may be due to alterations in the release of arginine vasopressin (7, 14). No study has addressed the impact of sex hormone administration on body fluid restoration following dehydration, but arginine vasopressin, measured during controlled rehydration, returns to pre-dehydration levels more slowly in women (follicular phase) compared to men (65). This slower restoration of arginine vasopressin is associated with greater fluid retention in women, suggesting the renal response to arginine vasopressin is unaffected by estrogen. These data also suggest a role for estrogen in the recovery of arginine vasopressin following dehydration. Prior to the present experimental series, no studies had evaluated systematically the impact of variable estrogen doses found in oral contraceptive pills on fluid regulation in women.

Our study was designed to test the hypothesis that oral contraceptive pills containing estrogen increase the thirst and arginine vasopressin response to plasma osmolality and plasma volume alterations during progressive dehydration to a greater degree than progestin-only pills. We expected that this increase in osmotic sensitivity would result in enhanced fluid intake and water retention during a subsequent *ad libitum* rehydration period.

In addition to the changes in arginine vasopressin, plasma concentrations of the sodium and water retention hormones, renin and aldosterone, increase during pregnancy (61), during estrogen-dominant oral contraception (8, 86) and during ovarian stimulation (61). Elevations in plasma estrogen concentration increase sodium retention (2, 64), due either to changes in body sodium distribution (2, 8), renal sodium reabsorption (16), or renin and aldosterone actions (61). During the mid-luteal phase of the menstrual cycle however, PRA and aldosterone increase only when ovulation occurs (47), indicating that a functioning corpus luteum (and the progesterone it secretes) is necessary to augment the renin-angiotensin-aldosterone system. In young, cycling women, the mid-luteal phase increase in endogenous progesterone is accompanied by an increase in estrogen, which may enhance the progesterone effect on the renin-aldosterone system (61).

The impact of estrogen on the renin-aldosterone system and sodium regulation has not been studied during dehydration, a time when both sodium and water retention systems are stimulated. In this study, we used a dehydration-rehydration protocol during combined (estradiol and progestin) or progestin-only oral contraceptive pills in order to distinguish specific estrogen effects on the sodium regulating hormones. The synthetic progestin, norethindrone, does not possess antimineralocorticoid properties (84), and the progestin-only pills contain no estrogen, which down-regulates estrogen receptors. Thus, these two oral contraceptive preparations differ significantly in their estrogenicity, and as such, may differ in their effects on sodium and water regulation. We hypothesized that the estradiol contained in combined oral contraceptive pills would slow the rate of electrolyte loss during dehydrating exercise, and enhance fluid and sodium retention during rehydration relative to control (follicular and luteal phase), and progestin-only pills. We further hypothesized that the greater fluid and sodium retention would be related to an estrogen-mediated stimulation of the renin-aldosterone system.

METHODS

Study design:

Ten women volunteered to participate in the dehydration experiments. Subjects were non smoking, healthy women, ages 21-31, with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, and had medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure, blood volume was determined by Evan's Blue dye dilution (procedures are described below). On the same day, following the blood volume assessment, maximal oxygen consumption (VO_{2peak},) was determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). The preliminary tests were all conducted in the follicular phase of the menstrual cycle.

Each woman served as her own control. Upon entering the study, the subjects were assigned (double-blind) to undergo experimental testing after four weeks of either continuous combined (estrogen/progestin) or progestin-only treatment (Fig.1). After completing the studies on one treatment protocol, subjects crossed over to the other treatment following a 4 week "washout" period.

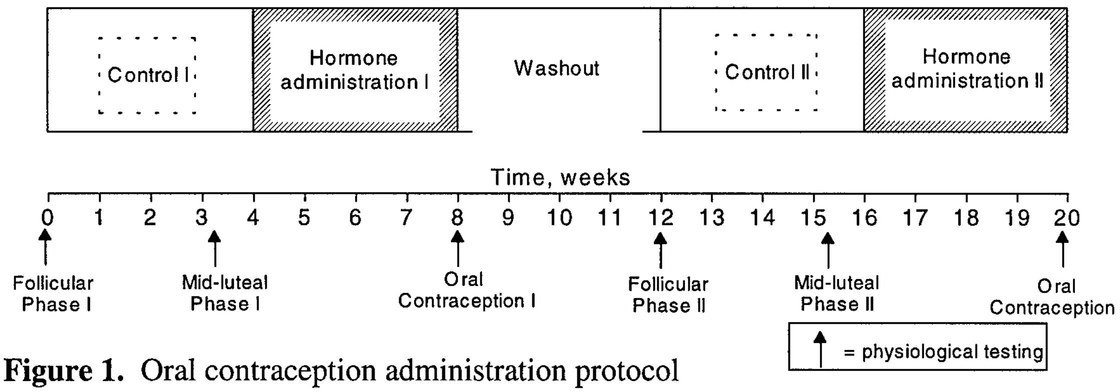

Figure 1. Oral contraception administration protocol

For estrogen/progestin combined treatment (OC E+P), subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. For progestin-only treatment (OC P), subjects received norethindrone, 1 mg/day. All studies were begun within 2 hours of the daily pill ingestion when peak serum hormone levels occur (11).

Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employed two dehydration baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle in the month preceding each oral contraception treatment. *The two control tests were completed during the month before each 28 day pill treatment (Fig 1).* The luteal phase was determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA) that accurately identify the luteinizing hormone peak. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the control (pre-exercise) blood sample.

Dehydration experiments

Volunteers arrived at the laboratory between 7:00 - 8:00 am, after having eaten only a prescribed low fat breakfast (~ 300 kcal). The subjects refrained from alcohol and caffeine for 12 h prior to the experiment. Blood volumes were un-manipulated prior to each of the experiments, although subjects were well hydrated by drinking 7 ml/kg body weight of tap water at home before arrival at the laboratory. Upon arriving at the laboratory, the subjects gave a baseline urine sample, were weighed to the nearest 10 g on a beam balance and then sat on the contour chair of a cycle ergometer in the test chamber (27°C, 30% rh) for 60 min of control rest. During the control period, an indwelling catheter was placed in an arm vein. Electrodes and blood pressure cuff were placed and resting blood pressure (Colin Medical Instruments Corp, Komaki, Japan), and heart rate (EKG) recorded at the end of the 60 min control period. At the end of the control period, a (20 ml) blood sample was drawn, control thirst tests (see below) administered and urine collected.

Hydration state was assessed from the specific gravity of the control (pre-exercise) urine sample (mean = 1.001).

Dehydration protocol

We have modified a Monark cycle ergometer by placement of an adjustable contour seat behind the pedals so that the subject was seated with legs nearly in a horizontal position. The exercise intensity was adjusted by changing the tension on the flywheel, and was normalized to each subject as determined by her individual VO_{2peak} test.

Following the control period, the chamber temperature was increased to 36°C. The subjects exercised at 50% maximal power output without fluids for 150 min, with 5 min rest periods every 25 min. Blood samples (10-20 ml) were drawn immediately prior to the rest periods at 60, 120 and 150 min during exercise. Thirst ratings were also assessed immediately prior to rest periods at 30, 60, 90, 120 and 150 min of cycling. During exercise, sealed absorbent patches (Sudormed, Santa Anna, California) were placed on the thigh, forearm, chest, back and forehead for 20-40 min periods for sweat collection. The sweat patch consisted of 4.7 x 3.1 cm filter paper, sealed and affixed to the skin with tegaderm. The area used for the patch was cleaned with deionized water prior to placement and wiped with a clean dry towel. After sampling, the patches were transferred to plastic screw-capped bottles. Local sweat rate was determined by patch weight increase (to 0.0001 g) from the dry weight per min on the skin. The fluid in the patches was collected by centrifugation with nylon MicroFuge centrifuge filter tubes and analyzed for sodium and potassium concentrations. Heart rate and blood pressure were assessed every 10 min throughout exercise. Body weight was determined at 60, 120 and 150 min of exercise, and urine samples were collected at the end of exercise. At the end of exercise, the chamber temperature was reduced to 27° C for the 3.5 h recovery period.

Following dehydration, volunteers rested for 30 min in a contour chair without access to fluids to allow the body fluid compartments to stabilize, after which, the subjects drank water *ad libitum* for 180 min. Heart rate and blood pressure were assessed every 10 min throughout stabilization and rehydration. Blood (10 ml) was sampled during the early period of rehydration (just prior to drinking, at 15 min of drinking) and at 30, 60, 120 and 180 min of rehydration (20 ml). Urine samples were collected at each 60 min of rehydration and body weight was measured every 60 min of rehydration.

All blood samples were analyzed for hematocrit, hemoglobin, total protein, osmolality, and the concentrations of creatinine, glucose, urea, sodium, potassium, and arginine vasopressin. The control and final blood samples were analyzed for 17-β-estradiol and progesterone. Blood samples at control, 60, 120 and 150 min of dehydration and at 0, 30, 60, 120 and 180 min of *ad libitum* drinking were also analyzed for the concentration of atrial natriuretic peptide, aldosterone, and plasma renin activity. All urine samples were analyzed for volume, osmolality, and sodium, potassium, and creatinine concentrations.

Blood sampling

All blood sampling was done via a 19 gauge Intracath catheter placed in an arm vein. Subjects were semi-recumbent during placement of the catheter and are seated for 60 min prior to sampling to ensure a steady state in plasma volume and constituents. Blood samples were separated immediately into aliquots. The first was analyzed for hemoglobin and hematocrit. A second aliquot was transferred to a heparinized tube, and third aliquot for the determination of serum sodium and potassium concentrations was placed into a tube without anticoagulant. All other aliquots were placed in tubes containing EDTA. The tubes were centrifuged and the plasma taken off the heparinized sample analyzed for sodium, potassium, osmolality, glucose, urea creatinine and aldosterone. The EDTA samples were analyzed for concentrations of arginine vasopressin and atrial natriuretic peptide and plasma renin activity.

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evan's blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing has occurred (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume was calculated from plasma volume and hematocrit concentration corrected for peripheral sampling.

Thirst ratings

The perception of thirst was assessed by asking the subject to make a mark on a line rating scale in response to the question, 'How thirsty do you feel now?'. The line is 175 mm in length and is marked 'not at all' on one end and 'extremely thirsty' at the 125 mm point. We tell subjects that they can mark beyond the 'extremely thirsty' point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (46) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and plasma osmolality during hypertonic saline infusion and dehydration in young volunteers.

Calculations

Total water loss due to dehydration was determined from body weight loss. Net fluid gain during rehydration was calculated by subtracting total urine loss from water intake, assuming that respiratory and sweat losses were negligible in the 27°C recovery condition. Electrolyte losses in sweat and urine during dehydration were calculated by multiplying the volume of water loss by the concentration of electrolyte in each fluid. Whole body sweat electrolyte concentration was calculated from sweat rate, local electrolyte concentration and body surface area using the following equation:

$$[E]_m = (0.07[E]_{fh}SR_{fh} + 0.36[E]_{tr}SR_{tr} + 0.13[E]_{fa}SR_{fa} + 0.32[E]_{th}SR_{th} / 0.07SR_{fh} + 0.36SR_{tr} + 0.13SR_{fa} + 0.32SR_{th})\ (77)$$

where the subscripts m, fh, tr, fa and th are whole body mean, forehead, trunk, forearm and thigh; [E] is electrolyte concentration (sodium or potassium, mEq/l), and SR is local sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$); and the constants 0.07, 0.36, 0.13 and 0.32 represent the percent distribution of body surface in the head, trunk, arms and legs, respectively. Total electrolyte loss from sweat was calculated by multiplying $[E]_m$ and total body sweat loss, calculated from the change in body weight during exercise. Electrolyte losses during rehydration were calculated by multiplying the volume of water loss by the concentration of electrolytes in the urine.

Changes in plasma volume were estimated from changes in hemoglobin (Hb) and hematocrit (hct) concentrations from the control (pre-exercise) sample according to the equation:

$$\% \Delta PV = 100\ [[(Hb_b)/(Hb_a)][(1\text{-}hct_a \cdot 10^{-2})]/[(1\text{-}hct_b \cdot 10^{-2})]] - 100$$

where subscripts a and b denote measurements at time a and control, respectively. Hemoglobin is measured in triplicate by the cyanomethemoglobin technique and hematocrit in triplicate by the microhematocrit method.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na+}) were calculated from the following equations:

$$FE_{H_2O} = (U_V/GFR) \cdot 100$$

$$FE_{Na+} = (U_V \cdot [Na^+]_u/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \cdot [Na^+]_s$$

where the subscripts f and u are glomerular filtrate and urine respectively, U_v is urine flow rate, and $[Na^+]_s$ is $[Na^+]_s$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Blood analysis:

Plasma, sweat and urine sodium and potassium are measured by flame photometry (Instrumentation Laboratory model 943), plasma osmolality by freezing point depression (Advanced Instruments 3DII), and plasma proteins by refractometry. Plasma glucose, urea and creatinine concentrations are determined by colorimetric assay (Sigma Diagnostic Products). Plasma renin activity, plasma concentrations of aldosterone, atrial natriuretic peptide, arginine vasopressin, 17-β-estradiol and progesterone are measured by radioimmunoassayIntra- and inter-assay coefficients of variation for the mid-range standard for AVP (4.52 pg/ml) were 6.0 % and 3.4 % (Immuno Biological Laboratories (IBL), Hamburg, Germany), for 17 β-estradiol (64.3 pg/ml) were 3.7 % and 4.0 % (Diagnostic Products, Los Angeles, CA), and for progesterone (3.7 pg/ml) were 2.1 % and 2.5 % (Diagnostic Products). The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

RESULTS

Combined oral contraceptive administration caused severe nausea in one woman, and she did not complete dehydration testing while on this pill, so all of her control data for OC E+P have also been excluded. In addition, one subject dropped out for personal reasons after completing two of the control tests, so all of her data was excluded from the analysis. This analysis compares the dehydration test responses of 9 women on OC P with their two control tests and 8 women on OC E+P with their control tests. There were no other significant adverse effects of oral contraceptive administration in any of the subjects.

Baseline (Pre-exercise). Pre-exercise body weight was similar for both phases of the menstrual cycle, and sex hormone administration (Table 1). Furthermore, $P_{[E_2]}$ and $P_{[P_4]}$ demonstrate that the subjects were tested in the early follicular phase and mid-luteal phase of the menstrual cycle during both trials. Finally, oral contraceptive administration suppressed the endogenous production of 17 β-estradiol and progesterone (Table 1).

Plasma osmolality was lower in both the luteal phase and following one month of OC E+P and OC P compared to the follicular phase (Fig. 2). Plasma glucose and urea concentrations were unaffected by menstrual phase or either oral contraceptive pill, indicating that the lower P_{Osm} was due to lower $S_{[Na+]}$ (Table 2). Pre-exercise $P_{[AVP]}$ and thirst were unaffected by phase of the menstrual cycle or by oral contraceptive administration (Tables 3 & 4). Hematocrit and [Hb] were elevated during the luteal phase (Table 3), indicating a contraction of plasma volume when compared to the follicular phase (-7.8 ± 2.6 %) and OC E+P (-8.0 ± 3.4 %) and OC P (-5.7 ± 1.6 %). Combined oral contraceptive pills increased plasma volume only slightly (3.2 ± 2.1 %) and OC P did not change plasma volume (-2.3 ± 2.5 %) compared to the follicular phase. There was no effect of menstrual phase or oral contraceptive treatment on plasma protein concentration (Table 3).

Basal PRA and $P_{[ALD]}$ were elevated in both luteal phase tests compared to the follicular phase tests and to the OC E+P and OC P tests (Figs 3 & 4, $P < 0.05$). In contrast, $P_{[ANP]}$ was greatest at baseline in the follicular phase tests and in the OC E+P test (Fig. 5). There were no differences between the OC E+P and OC P tests in PRA, $P_{[ALD]}$ or $P_{[ANP]}$ at baseline. Pre-exercise urine flow, GFR, free water and osmolar clearances and renal electrolyte excretion were similar within subjects prior to each exercise test (Tables 5 & 6).

Heart rate and blood pressure were similar at baseline and dehydration within the follicular and luteal phase tests so the combined mean of the two series is given for the baseline values and for the dehydration tests. Baseline heart rate and mean blood pressure were unaffected by menstrual phase or by oral contraceptive treatment (Tables 7A and 7B).

Exercise. At the end of 150 min of exercise at 36°C, the women lost the same amount of body water through sweating in the early follicular phase (1.5 ± 0.2 and 1.5 ± 0.1 kg), the mid-luteal phase tests (1.4 ± 0.1 and 1.4 ± 0.1 kg), the OC E+P test (1.5 ± 0.1 kg) and the OC P test (1.3 ± 0.1 kg). Heart rate increased to similar levels during dehydrating exercise in the follicular and luteal phase tests and during the OC P test, but this increase was attenuated during the OC E+P test (Tables 7A and 7B). Mean blood pressure did not change during dehydration in any of the experimental conditions.

Exercise increased P_{Osm} and $P_{[AVP]}$, and decreased plasma volume similarly during the follicular and luteal phases, and during OC E+P and OC P (Fig. 2 & Table 3). Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{Osm}, with r values ranging from 0.82 to 0.98. The abscissal-intercepts of the linear $P_{[AVP]}$-P_{Osm} relationship, or "theoretical osmotic threshold" for AVP release, was significantly lower in the mid-luteal phase and OC E+P than in the follicular phase (Table 1, $P < 0.05$). The slopes of this relationship were unaffected by menstrual phase or oral contraceptive pills. Figure 6 shows the downward shift in the linear $P_{[AVP]}$-P_{Osm} relationships during OC E+P and when $P_{[E_2]}$ and $P_{[P_4]}$ were increased during the luteal phase.

The data in Table 4 indicate that thirst increased similarly during dehydration in all conditions. Linear regression analysis of the individual subjects' P_{Osm} and thirst responses indicated significant correlations, with r values ranging from 0.73 to 0.99. Osmotic thirst stimulation was unaffected by menstrual phase and there were no effects of oral contraceptives on the slope or abscissal intercept of this relationship (Table 1).

Plasma renin activity, $P_{[ALD]}$ and $P_{[ANP]}$ increased during exercise in all conditions, with luteal phase values for PRA and $P_{[ALD]}$ remaining above the follicular phase, OC E+P and OC P (Figs. 3 & 4). For $P_{[ANP]}$, neither menstrual phase nor oral contraceptive treatment affected the magnitude of the exercise-induced increases (Fig. 5). Sweat sodium loss was greatest during exercise in the follicular phase tests (56.3 ± 7.0 and 59.4 ± 9.2 mEq, $P < 0.05$), but was similar between the luteal phase tests (45.2 ± 9.1 and 46.5 ± 7.8 mEq) compared to the OC E+P (47.1 ± 10.7 mEq) or OC P (46.7 ± 8.8 mEq) tests. Sweat potassium loss was unaffected by menstrual phase or oral contraception administration (5.32 ± 0.71, 5.92 ± 0.59 and 5.35 ± 0.42 mEq for follicular and luteal phase tests and the OC E+P test, respectively) and (5.42 ± 0.57, 4.47 ± 0.39 and 4.86 ± 0.62, for follicular and luteal phase tests, and the OC P test, respectively). Renal sodium excretion was increased during exercise in all conditions, and this increase was greatest during the follicular phase tests (Table 6, $P < 0.05$). The cumulative sodium (sweat + urine) loss was greatest during the follicular phase tests compared to the luteal phase tests, and the OC E+P and OC P tests (Fig. 7).

Rehydration. Ad libitum fluid intake was similar by the end of the 180 min of rehydration on all six experimental test days (Fig 8). At 180 min of *ad libitum* drinking, subjects had restored 41 ± 5 and 40 ± 10 % (follicular phase), 42 ± 7 and 39 ± 6 % (luteal phase), 38 ± 11 % (OC E+P) and 39 ± 7 % (OC P) lost during dehydration. Plasma osmolality was higher throughout the rehydration period in the follicular phase compared to the luteal phase, OC E+P and OC P tests (Fig. 2). Recovery of $P_{[AVP]}$ and thirst was rapid following the beginning of *ad libitum* drinking, and similar during all rehydration tests (Tables 3 & 4).

For the entire rehydration period, area under the curve for PRA (Fig. 3) was lower during the follicular phase tests ($P < 0.05$) compared to the luteal phase tests and the OC E+P test. Area under the curve for $P_{[ALD]}$ (Fig. 4) was significantly greater in the luteal phase tests compared to the follicular phase tests, and compared to the OC P test ($P < 0.05$). There were no effects of oral contraceptives or menstrual phase on $P_{[ANP]}$ during rehydration (Fig. 5).

Urine flow and renal free water clearance were lower at the end of drinking during OC E+P than in both the follicular and the luteal phase tests (Table 6, $P < 0.05$). Cumulative urine loss was greatest (Fig 8, $P < 0.05$) during the follicular phase relative to the other conditions, although overall fluid balance (i.e. fluid intake - urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration. During rehydration, electrolyte excretion was unaffected by menstrual phase or oral contraceptive administration (Table 6). However, because of the greater exercise sodium excretion during the follicular phase, cumulative sodium loss (exercise + rehydration) was greatest during the follicular phase (Fig. 7).

DISCUSSION

Osmotic regulation of AVP and fluid balance

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it was demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive (21, 83), but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids.

Estrogen most likely modulates osmotic AVP regulation via its action within the central nervous system, due to the fact that it readily crosses the blood-brain barrier. Studies in lower animals have demonstrated that estrogen acts directly on estrogen-binding neurons in the hypothalamus (3, 7, 19, 57), thereby affecting synthesis and release of AVP. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (57), and osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the supraoptic nucleus of brain slices of ovariectomized rats (7). Estrogen may also modulate hypothalamic AVP release indirectly through catecholaminergic (35) and/or angiotensinergic (73) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [^{3}H]-labeled estradiol, Heritage et al. (35) identified estradiol binding sites in the nuclei of catecholamine neuronal systems, as well as the presence of catecholamine nerve terminals surrounding estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (20) noted parallel changes in brain norepinephrine and AVP in normally cycling rats, and that ovarian steroids modulated norepinephrine turnover in the paraventricular nucleus, indicating that estrogen may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (73).

Peripheral mechanisms for the estrogen effect on osmotic stimulation of AVP are unlikely to participate in the response. For example, plasma volume reduction, such as that which occurred during the mid-luteal phase, could have contributed to the lower P_{Osm} threshold for AVP release because plasma volume is a potent AVP stimulus. However, this mechanism seems unlikely because the luteal phase-plasma volume contraction was not associated with a fall in blood pressure. Further, AVP was also upregulated during OC E+P administration, during which changes in pre-dehydration plasma volume did not occur. Atrial natriuretic peptide has also been shown to suppress the osmotically-induced rise in AVP (17) but the follicular phase and OC E+P were both associated with greater plasma atrial natriuretic peptide levels, and had vastly different vasopressin responses.

Despite the lower osmotic threshold for AVP, there were no changes in water intake, which matched urine output, indicating a new set point for fluid regulation in the presence of high plasma estrogen levels. In addition to reducing the osmotic threshold for AVP release, estrogen may alter the renal sensitivity to AVP by attenuating its antidiuretic action. There is evidence that estrogen modulates AVP action in the rat collecting duct (20) at the receptor level (75). Our observation that the greater osmotic secretion of AVP in the mid-luteal phase of the menstrual cycle was not accompanied by increased water retention is consistent with these findings. In contrast, we also found that renal C_{H_2O} was reduced during combined estrogen and progesterone administration despite similar $P_{[AVP]}$. Moreover, estrogen administration to postmenopausal women has been shown to increase renal concentrating response (U_{Osm}/P_{Osm}) to hypertonic saline infusion, despite similar $P_{[AVP]}$ responses (64). Future studies that determine the renal dose-response relationship of AVP are necessary to determine the impact of estrogen and progesterone on the kidney.

Finally, combined estrogen and progestin oral contraception administration increased plasma volume by as much as 12.4 % relative to the mid-luteal phase of the menstrual cycle. Estrogen-mediated increases in plasma volume are consistent with earlier findings in postmenopausal (2, 64) and young women (8, 25). The estrogen-mediated plasma volume expansion is not always accompanied by changes in water retention, and the mid-luteal phase plasma volume contraction not always associated with greater urine loss. A number of earlier studies demonstrated that high plasma levels of estrogen and progesterone alter Starling forces to favor protein and fluid movement out of the vasculature (41, 42, 79, 80). Therefore, these steroids may have their primary effect by altering body water distribution, rather than body water balance.

Sodium Regulation

Our experimental design enabled us to isolate estrogen effects on the renin-aldosterone system because norethindrone administered alone and with estradiol did not exhibit antimineralocorticoid properties. Our major finding was that neither estrogen dominant, nor progestin-only oral contraceptives increased PRA or $P_{[ALD]}$; rather, we found only the high endogenous estrogen and progesterone present in the luteal phase enhanced PRA and $P_{[ALD]}$. Sodium loss (sweat + urine) was attenuated during dehydration in the luteal phase and during OC E+P and OC P, but these losses were not necessarily associated with increases in the sodium regulation hormones indicating that norethindrone inhibits sodium loss, but through a mechanism other than the renin-aldosterone system.

Combined oral contraceptive pills deliver pharmacological levels of ethinyl estradiol (10), which is almost identical in structure to the most biologically active form of endogenous estrogen, 17 β-estradiol, although with four times the potency (44). Our data do not support a role for estrogen in the stimulation of the renin-aldosterone system because OC E+P did not augment renin or aldosterone. Norethindrone, a progestational derivative of testosterone, differs in structure from endogenous progesterone. Endogenous progesterone inhibits aldosterone-dependent sodium reabsorption at distal sites in the nephron and produces a transient natriuresis (50) followed by a compensatory stimulation of the renin-aldosterone system (47, 76, 86). In contrast, norethinedrone does not possess antimineralocorticoid properties because neither OC E+P nor OC P led to increases in PRA or $P_{[ALD]}$. Nonetheless, administration of norethindrone, with and without estrogen, enhanced sodium retention, suggesting this synthetic form of progesterone may act directly on the renal tubules.

Our data extend earlier findings demonstrating plasma volume contraction concomitant with enhanced PRA and $P_{[ALD]}$ during the mid-luteal phase of the menstrual cycle at rest, exercise and heat exposure (70, 72). The luteal phase was characterized by a baseline plasma volume contraction of ~220 ml compared to the follicular phase and of ~283 ml compared to OC E+P. Basal plasma sodium content also decreased in the luteal phase (377 ± 22, 340 ± 26, 388 ± 24, 368 ± 22 mEq, $P < 0.05$, for the follicular and luteal phases, and OC E+P and OC P, respectively). However, although plasma volume and sodium content contraction are powerful stimuli to the renin-aldosterone system, they were not accompanied by changes in blood pressure so may not have contributed directly to the increases in PRA and $P_{[ALD]}$.

Progesterone and/or estrogen may modulate plasma volume and sodium content through inhibition of ANP release from cardiac myocytes. Atrial natriuretic peptide plays a role in the homeostatic feed back system that regulates sodium balance, that is, sodium- and volume-retaining stimuli increase ANP, which, in turn, antagonizes renin and aldosterone (10, 53, 62). Progesterone administration can suppress $P_{[ANP]}$ (85), so increases in circulating endogenous progesterone may inhibit ANP release during the luteal phase, and thus reduce sodium excretion. Furthermore, there is evidence that progesterone interferes with the inhibitory effects of ANP on aldosterone secretion (49, 53), suggesting that progesterone may enhance $P_{[ALD]}$ not only by attenuating ANP release, but by reducing the inhibitory actions of $P_{[ANP]}$ on the adrenal cortex. In our investigation, combined oral contraceptive pills increased $P_{[ANP]}$ at baseline and during dehydration, while OC P reduced $P_{[ANP]}$ to luteal phase levels, suggesting the estrogen in OC E+P may have modified a progesterone-modulated $P_{[ANP]}$ inhibition during dehydration. Alternatively, estrogen receptors are found in cardiac myocytes (74), so estradiol may stimulate ANP release directly.

Our findings also suggest that estrogen impacts water and protein distribution in the body. Despite the plasma volume contraction in the luteal phase, total protein concentrations were unchanged during the luteal phase tests, indicating that both water and protein left the vasculature. Indeed, circulating plasma proteins (183.7 ± 11.3, 175.6 ± 10.7, 192.6 ± 13.1 and 184.8 ± 10.6 g, combined means for the follicular and luteal phase tests, and OC E+P and OC P, respectively) were lowest in the luteal phase tests compared to all other test conditions. Estrogen-mediated changes in body water and protein distribution are consistent with earlier studies in which the level of plasma volume expansion could not account for level of increases in overall body water retention (64). For example, during hypertonic saline infusion in estrogen-treated postmenopausal women, body water retention was increased by 31%, but plasma volume was unchanged (64). Finally, earlier studies have demonstrated that estrogen and/or progesterone alter transcapillary fluid dynamics to favor fluid and protein movement into the extravascular (interstitial) compartment (79, 80).

Any estrogen- or progesterone- mediated changes in transcapillary fluid dynamics may also have occurred via ANP. Atrial natriuretic peptide has important effects on body fluid dynamics, and may contribute to plasma volume regulation by inducing extravascularization (31, 55). Low-dose ANP infusions (to ~150 pg/ml) augment the capillary filtration coefficient (31), probably due to ANP-mediated changes in protein permeability. The increase in plasma protein permeability allows plasma proteins to escape from the circulation into the interstitial fluid, decreasing the rise in the colloid osmotic pressure of the microvasculature, opposing fluid reabsorption from the interstitium, and thus causing the extravascular efflux of proteins and fluid. Although estrogen and progesterone may increase ANP release, or impact its actions, the extent to which these hormones interact with ANP and modulate body water distribution has not been determined.

We used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation during dehydration and a subsequent rehydration period. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

CONCLUSIONS

We found that normally cycling young women have a reduction in the osmotic threshold for AVP release during the mid-luteal phase of the menstrual cycle (i.e. when estrogen and progesterone peak). Further, the osmotic threshold for AVP release is lowered during administration of oral contraceptives containing estrogen, but this reduction in threshold did not occur during progestin-only oral contraceptive use. Previously it was demonstrated that estrogen and progesterone upregulate thirst and AVP responses to an osmotic drive (21, 83), but the upregulation could not be attributed to specific estrogen or progesterone effects. Our data extend these early findings by demonstrating a reduction in the P_{Osm} threshold for AVP release during estrogen-containing oral contraception administration. This threshold shift did not occur when the oral contraceptive contained only progestin, implicating estrogen as the hormone mediating the changes in AVP regulation. Because the water intake during the rehydration phase was similar in all our studies, regardless of menstrual phase or oral contraceptive treatment, we are able to conclude that an elevated circulating estrogen alters the body tonicity around which the body regulates fluids.

Regarding sodium regulation, we used oral contraceptive pills to evaluate estrogen effects on the renin-aldosterone system and sodium regulation. During dehydration, we found that sodium loss was attenuated during the luteal phase and during administration of oral contraceptives containing estradiol and progestin, but these effects on sodium regulation were not mediated through the renin-aldosterone system. While estrogen does not appear to have direct effects on the renin-angiotensin-aldosterone system, this hormone may impact sodium regulation by modifying a progesterone-modulated inhibition of ANP release. In addition, the changes in sodium regulation may also have been influenced by the changes in resting plasma volume and sodium content.

Reliability of fluid regulation hormones

INTRODUCTION

Despite the continued study of changes in the fluid and sodium regulating hormones, there were no studies examining their stability, or reliability within a given phase, over the course of two or more menstrual cycles. Differences in reported plasma concentrations of these hormones across different menstrual cycles can be affected by natural variations within a woman, by inaccurately choosing the appropriate day of each phase of the menstrual cycle to conduct physiological testing, by differences in water and/or sodium intake, or by difficulty with the hormone analysis techniques. We determined the reliability of the fluid and sodium regulating hormones, aldosterone, renin, arginine vasopressin and atrial natriuretic peptide, at rest and in response to dehydrating exercise over two menstrual cycles. Accordingly, we tested the reliability of the fluid regulating hormones in our subjects on the above-described dehydration testing days: twice during the early follicular phase (when estrogen and progesterone are low) and twice during the mid-luteal phase of the menstrual cycle (when estrogen and progesterone are high).

METHODS

Subjects were nine non-smoking, healthy women, ages 21-3. All subjects were interviewed about their medical history, and had medical and gynecological examinations before admission to the study. During the month preceding the first dehydration/rehydration exposure maximal oxygen consumption (VO_{2peak},) was determined with an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA). This preliminary test was conducted in the early-follicular phase of the menstrual cycle.

The study design employed four dehydration experiments, two conducted in the early-follicular phase (2-4 days (4 ± 1) after the beginning of menstrual bleeding) and two in the mid-luteal phase of the menstrual cycle (20-25 days (22 ± 2 days) after the start of menstrual bleeding). Specifically, for the mid-luteal phase tests, the subjects were tested between days 7-10 following the LH peak, and therefore approximately 6-9 days after ovulation. The dehydration protocol is described on pages 3-5 of this Progress Report (See "Dehydration experiments").

Statistical Analysis. Pearson's Product Moment Correlation on individual data was used to assess the slope and abscissal intercepts of the P_{Osm} - $P_{[AVP]}$ relationship during dehydration (21). The within-phase reliability of our most important dependent variables, fluid regulating hormones and osmotic regulation of AVP, measured at rest, dehydration and rehydration, was determined with Cronbach's α, assuming a value ≥ 0.80 as a acceptable level of reliability (9). Areas under the curve (AUC, trapezoid method) were calculated during the rehydration period for PRA, $P_{[ALD]}$ and $P_{[ANP]}$, and their reliability determined within a given menstural using Cronbach's α. We used repeated measures ANOVA models, followed by Bonferoni's *t*, to test differences in the dependent variables both within and between menstrual phases. Data were analyzed using BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA), and expressed as mean ± SEM.

RESULTS

Within-phase reliability.

Early follicular phase. Within the follicular phase, there were no significant differences between the means of any of the variables during rest, dehydration and rehydration (Table 8). In fact, most of the hormonal responses demonstrated high reliability, attaining a Cronbach's α greater than 0.80 (Table 9). However, with the exception of $P_{[ANP]}$, none of the resting values of the fluid regulating hormones attained sufficiently high Cronbach's α to be considered reliable (Table 9). Reliability was improved following dehydrating exercise for $P_{[AVP]}$ and PRA; although it remained low for $P_{[ALD]}$ ($\alpha = 0.66$) and remained high for $P_{ANP]}$ ($\alpha = 0.90$). During dehydration, both the slope and abscissal intercept of the P_{Osm} - $P_{[AVP]}$ relationship were highly reliable within the follicular phase, attaining Cronbach's α of 0.96 and 0.90, respectively. Again, $P_{[AVP]}$, $P_{[ALD]}$ and PRA were not reliably reproduced during rehydration, while Cronbach's α for $P_{[ANP]}$ was 0.93. Plasma estrogen concentration was highly reproducible within the follicular phase tests, attaining Cronbach's α of 0.85, but $P_{[P_4]}$ attained a Cronbach's α value of only 0.62 between tests in the follicular phase.

Mid-luteal phase. Similar to the follicular phase, there were no differences mean hormonal concentrations at rest, after dehydration or during rehydration within the mid-luteal phase (Table 8). Again, resting values for $P_{[AVP]}$, $P_{[ALD]}$ and PRA were not highly reproducible between the two mid-luteal phase tests (Table 9). Reliability for $P_{[ANP]}$ was greater compared to the other fluid regulating hormones, at rest and during exercise and rehydration, and again, despite high levels of reliability for osmotic regulation of AVP (Table 9), resting and rehydration levels of $P_{[AVP]}$ were not consistently correlated within the luteal phase tests. In contrast to the follicular phase however, both $P_{[E_2]}$ and $P_{[P_4]}$ were highly reliable between the two luteal phase tests, yielding Cronbach's α values of 0.93 and 0.93, respectively.

CONCLUSIONS

We examined the within-phase reliability of plasma concentration of fluid and sodium regulating hormone concentrations between two separate menstrual cycles at rest and in response to dehydration during the early follicular and mid-luteal phases. Resting and recovery plasma concentrations of AVP, aldosterone and PRA were not reproducible within each of the different menstrual phases; however, there were no statistical differences between the means of any of these hormone concentrations indicating that the within-subject inconsistency remains undetected when only the means are tested or reported. Nonetheless, our data indicate that between phase differences in the hormone concentrations far exceed the variability within the phases, and therefore the low within-phase reliability does not prevent the detection of menstrual phase-related changes in these variables. In contrast, however, $P_{[AVP]}$, PRA and $P_{[ANP]}$ responses to dehydrating exercise were highly reliable within each menstrual phase indicating that hormonal responses to stress are more consistent in spite of the variability in baseline values.

Responses to technical issues regarding the Progress Report from 1997:

(1) We have attempted to clarify the timing the experiments in the text (Page 3) with the following *"Because sex hormones vary across the menstrual cycle, some variation in the dependent variables over the course of the menstrual cycle may exist. Therefore, the study design employed two dehydration baseline studies, carried out in the early-follicular phase (2-5 days after the beginning of menstrual bleeding) and mid-luteal phase of the menstrual cycle in the month preceding each oral contraception treatment."* In addition, we have added a figure to illustrate the protocol (See Figure 1).

(2) The letter "h" was changed to "hours" after the "2" in this sentence to clarify the timing on page three. The sentence now reads "All studies were begun within 2 hours of the daily pill ingestion when peak serum hormone levels occur (11)."

(3) The other major technical issue raised by the reviewers was regarding the long-term effect on these hormones on the variables we have measured. Of course we cannot answer this question from our data because our treatment only extends one month (28 days). However, there are a few studies that demonstrate that the effects on body water expansion (8), and body water distribution (79, 80) last least as long as 6 months (79, 80) to one year (8). It has also been demonstrated that 2 months of oral contraceptive treatment leads to increases in blood volume at rest, as well as increases in stroke volume and cardiac output during exercise (43).

Protocol B: Sex Hormone Effects on Thermoregulation during Exercise in the Heat.

The regulation of body temperature in humans is known to interact with systems that regulate volume and osmotic pressure of the extracellular fluid (48). Blood volume expansion improves the efficiency of cardiovascular and thermoregulatory responses during physical activity. In a study in which we manipulated blood volume in young men by ~ 9% of normal (27), after 30 min of moderately heavy exercise in the heat, internal temperature rose to 38.6°C in the control condition, to 38.3°C in the volume-expanded condition and the 38.9°C in the volume-contracted condition. The likely reason for the dependence of heat transfer on absolute blood volume during exercise in the heat is that the ability of the heart to pump blood to the skin, and therefore provide increased convective heat transfer from the body core to the skin, is a function of preload. When blood volume is expanded, cardiac stroke volume increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously, where heat transfer takes place. Conversely, blood volume contraction results in a gradual fall in preload during exercise (81), a reduction in cardiac output and an associated increase in skin vascular resistance at any internal temperature, explaining the decrease in heat transfer. These observations imply that a reflex sensitive to changes in the filling pressure of the heart influences the distribution of blood flow (and thus the resident blood volume) and thereby affects the body's ability to dissipate the excess heat produced during exercise.

The temperature threshold for the onset of a thermoregulatory effector response, i.e. sweating and peripheral vasodilation, is defined as the core temperature above which the effector response is greater than that of baseline. A shift in the core temperature threshold is often referred to as a change in the set-point for temperature regulation (71). A reduction in the set-point for temperature regulation secondary to blood volume expansion has profound effects on performance of physical activity in the heat because core temperature is maintained at a lower level and strain on the cardiovascular system is reduced. Conversely, dehydration (plasma volume loss) elevates exercise core temperature (58, 60) and decreases exercise tolerance (59, 60).

Estrogen may alter the threshold for thermoregulation during exercise in the heat. Core temperature responses to passive heating and exercise in heat are reduced during the follicular phase of the menstrual cycle, the cycle phase characterized by rising estrogen levels (36, 37, 39, 54). Haslag and Hertzman (34) demonstrated that the onset of thermoregulatory sweating during whole body heating occurred at a lower core temperature in women during their follicular phase. Stephenson and Kolka reported lower core temperature thresholds of both sweating (39, 68, 69) and cutaneous vasodilation in a hot environment (68, 69) during the follicular phase. No studies have assessed directly the effect of oral contraceptives on thermoregulatory responses during exercise in the heat, but the thresholds for the onset of sweating and vasodilation were reduced by 0.47°C and 0.48°C, respectively following 2 weeks of estrogen replacement therapy in post-menopausal women (78) and is reduced during long term estrogen therapy (12). The plasma volume expansion that is an outcome of estrogen administration (78), and the follicular phase of the menstrual cycle (70) may play an important role in the improved thermoregulation in the presence of high plasma levels of estrogen. This phase of the study was designed to determine the impact of estrogen-induced plasma volume expansion on thermoregulatory responses to exercise in the heat.

METHODS:

Study design:

Subjects were nine healthy, nonsmoking women (age 25 ± 1 y, range 22-31 y), with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, had medical and gynecological examinations and provided written confirmation of a negative Papanicolaou smear within one year of being admitted to the study. During the month (early follicular phase) preceding the first heat stress experiment, resting plasma volume was determined with Evan's blue dye dilution (see below) and peak oxygen consumption (VO_{2peak},) was determined from an incremental cycle ergometer test, using an automated metabolic cart (Sensor Medics Corp, Yorba Linda, CA).

Each woman participated in six baseline experiments, four baseline heat stress tests (2 in the follicular and 2 in the luteal phase of the menstrual cycle), and one heat stress test while taking each type of oral contraceptive (two total). Estrogen and progesterone vary across the menstrual cycle, so the study design employed a heat stress test conducted in the early-follicular phase, 2-4 days after the beginning of menstrual bleeding (low estrogen and progesterone), one for each pill treatment, and one conducted in the mid-luteal phase, 7-9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA). We report on two control tests per subject (one follicular, one luteal phase). Not all subjects had two ovulatory cycles. When only one ovulatory cycle occurred, data from only that menstrual cycle was used in the analysis. When both menstrual cycles were ovulatory, we randomly chose the first cycle to use in the analysis. When neither cycle was ovulatory, the subject was excluded from further analysis. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the pre-exercise blood sample prior to undertaking the temperature regulation protocol.

After completing the baseline heat stress tests, the subjects again performed heat stress protocols after four weeks of either continuous combined (estrogen-progestin, OC E+P) or progestin-only (OC P) oral contraceptive treatment (random assignment). Following a 4-week "washout" period, the subjects crossed-over to the other pill treatment. During OC E+P, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin, norethindrone daily. During OC P treatment, subjects received 1 mg/day of the progestin, norethindrone.

Heat stress tests

Volunteers arrived at the laboratory between 7:00 - 8:00 am, after having eaten only a prescribed low fat breakfast (~ 300 kcal). The subjects refrained from alcohol and caffeine for 12 h prior to the experiment. Blood volumes were not manipulated prior to any of the experiments, although subjects pre-hydrated by drinking 7 ml/kg body weight of tap water at home before arrival at the laboratory. Upon arriving at the laboratory, each subject gave a baseline urine sample, was weighed to the nearest 10 g on a beam balance, and was instrumented for the measurement of cardiac output (see below). The subject then sat on the contour chair of a semi-recumbent cycle ergometer in the test chamber (27°C, 30% rh) for 45 min of control rest. During the control period the subject was instrumented for the measurement of esophageal and skin temperatures, sweat rate, and blood pressure. An indwelling catheter (21ga) was inserted into an arm vein for blood sampling, and a heparin block (20 U/ml) maintained catheter patency. Subjects were semi-recumbent during placement of the catheter and were seated for 45-min prior to sampling to ensure a steady state in plasma volume and constituents. Resting blood pressure (Colin Medical Instruments Corp, Komaki, Japan), heart rate (EKG) and cardiac stroke volume were recorded at the end of the 45-min control period. At the end of the control period, a blood sample (12 ml) was drawn and urine collected. Hydration state was assessed from the specific gravity of the pre-exercise urine sample (mean = 1.002 ± 0.001).

Following 20 min of control measurements, the chamber temperature was increased to 35°C and the subject sat quietly for 20 min of passive heating. Measurements were made of arterial blood pressure every 10 min, cardiac output at 15 min, esophageal temperature and mean skin temperature continuously. At the end of the passive heating, another blood sample (12 ml) was drawn.

Immediately following passive heating, the subjects exercised on a recumbent bicycle at 60 % of their individual VO_{2peak} for 40 minutes. The subjects exercised with a fan positioned directly in front of the bike, with a fan speed 1.6 m/s to promote continuous evaporative sweating (1). Blood pressure was measured every 10 min, esophageal temperature and skin temperatures were monitored continuously and cardiac output estimates were obtained at 15 and 35 min during exercise. Sweating rate was also determined continuously throughout exercise. Blood was sampled at 10, 20 and 40 min of exercise.

Measurements

Body core temperature (T_{es}) was measured continuously from an esophageal thermocouple at the level of the left atrium. Skin temperatures were measured on the forehead, chest, upper arm, lateral flank, thigh and calf. T_{es} and T_{sk} were collected at a rate of 5 data points/s. Data were stored in a computer through an analog-to-digital converter system (ACRO 931, Daisylab, National Instruments, Austin, TX) as a mean value of every 30 seconds. Mean skin temperature was calculated from the following equation which takes into consideration surface area (32) and the thermosensitivity of each skin area (51).

$$T_{sk} = 0.10\ T_{ch} + 0.21\ T_{fh} + 0.28\ T_{ab} + 0.18\ T_{ua} + 0.15\ T_{th} + 0.18\ T_{ca}$$

Where subscripts refer to mean skin (sk), chest (ch), forehead (fh), abdomen (ab) upper arm (ua), thigh (th) and calf (ca).

An automatic dew-point sensor enclosed in a ventilated, plexiglas capsule was placed on the forearm and secured with surgical glue to determine sweating rate (29). Cardiac stroke volume was measured noninvasively by impedance cardiography (Minnesota Impedance Cardiograph, Model 304B), with two silver tape electrodes placed around the neck and two around the torso. The distance between the inner tapes was measured and made identical for all four experiments. Cardiac stroke volume was calculated using the equation of Kubicek et al. (40) and was averaged (ensemble averaging) over 25 seconds.

All blood samples were analyzed for hematocrit (Hct) and the concentrations of hemoglobin (Hb), total protein (TP), plasma osmolality (P_{Osm}) and serum concentrations of sodium ($S_{[Na^+]}$) and potassium ($S_{[K^+]}$). The control blood samples were also analyzed for 17 β-estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$).

Blood and urine analysis:

An aliquot (1 ml) was removed for immediate assessment of Hct, [Hb], and [TP] in triplicate by microhematocrit, cyanomethemoglobin and refractometry respectively. A second aliquot was transferred to a heparinized tube, and a third aliquot was placed into a tube without anticoagulant for the determination of $S_{[Na^+]}$and $S_{[K^+]}$. All other aliquots were placed in chilled tubes containing EDTA for analysis of $P_{[E_2]}$ and $P_{[P_4]}$. The centrifuged samples were frozen immediately at stored at –80°C until analysis. All urine samples were analyzed for volume, osmolality, creatinine, sodium and potassium concentrations.

Serum and urine sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory, Model 943). Plasma and urine osmolality (U_{Osm}) were assessed by freezing point depression (Advanced Instruments 3DII). Plasma concentrations of , $P_{[E_2]}$ and $P_{[P_4]}$ were measured by radioimmunoassay. Intra- and inter-assay coefficients of variation for the mid-range standard for $P_{[E_2]}$ (58 ± 4 pg/ml) were 15 % and 4 % (Diagnostic Products, Los Angeles, CA), for $P_{[P_4]}$ (1.7 pg/ml) were 14 % and 6 % (Diagnostic Products).

Blood volume

Absolute blood volume was measured by dilution of a known amount of Evan's blue dye dilution. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the first 10 min. Blood volume was calculated from plasma volume and hematocrit concentration corrected for peripheral sampling (30).

Changes in plasma volume (PV) were estimated from changes in Hct and [Hb] from the control (pre-exercise) sample according to the equation:

$$\% \Delta PV = 100 [[(Hb_b)/(Hb_a)][(1-Hct_a \cdot 10^{-2})]/[(1-Hct_b \cdot 10^{-2})]] - 100$$

in which subscripts a and b denote measurements at time a and control, respectively. We used this equation to calculate both changes from baseline during exercise within a given experimental day, as well as changes between each experimental day versus the follicular phase.

Electrolyte losses in urine were calculated by multiplying the volume of water loss in each fluid by the concentration of the electrolyte within the fluid [Not reported]. Total body sweat loss, calculated from the change in body weight during exercise.

Statistics. Prior to statistical treatment, the independent variable (time) was partitioned into 5-min bins. Within each subject, the dependent variables were averaged for every other bin, so that each averaged time period was separated by a five-min partition. To determine individual T_{es} thresholds for the onset of sweating, each subject's sweating rate (30 second value) was plotted as a function of T_{es} (30 second value) during exercise, and the T_{es} threshold for sweating (i.e. the T_{es} above which the effector response is greater than that of baseline) was determined by two independent investigators. The average estimate was used for analysis, and the estimates had an interrater reliability of 0.95. We used repeated measures ANOVA models, followed by Bonferoni's *t*, to test differences in the sweating threshold and slopes due to menstrual phase or oral contraceptive treatment (18). Based on an alpha level of 0.05 and a sample size of 8, our beta level (power) was ≥ 0.80 for detecting effect sizes of 0.28°C. Data were analyzed using BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA) and expressed as mean ± SEM.

RESULTS

Subject Characteristics

Two subjects did not have a luteal phase progesterone peak, so their data were excluded from further analysis. Therefore, all statistical analyses were performed on the remaining seven subjects and only their data are presented. On the pre-testing orientation day, the subjects weighed 53.0 ± 3.1, were 162 ± 3 cm tall, and their plasma and blood volumes were 2642 ± 258 ml and 74.3 ± 6.6 ml/kg, respectively, and their VO_{2peak} was 34.8 ± 2.1 ml/kg on the recumbent bicycle ergometer. Plasma levels of 17 β-estradiol and progesterone were consistent with expected values during the early follicular and mid-luteal phases of the menstrual cycle, and were suppressed during oral contraceptive treatment (Table 10).

Pre-exercise. During thermoneutral rest, T_{es} was greater during OC P compared to the follicular phase and OC E+P and was also greater during the luteal compared to the follicular phase (Table 1, $P < 0.05$). Mean T_{sk} was not affected by menstrual phase or oral contraceptive treatment. Relative to the follicular phase, plasma volume was decreased by (–3.8 ± 2.2 %, (-115 ml) during the luteal phase, increased by 7.3 ± 3.4 % (190 ml) during OC E+P treatment, but unchanged (–0.7 ± 1.8 ml (-36 ml) during OC P treatment (Fig 9).

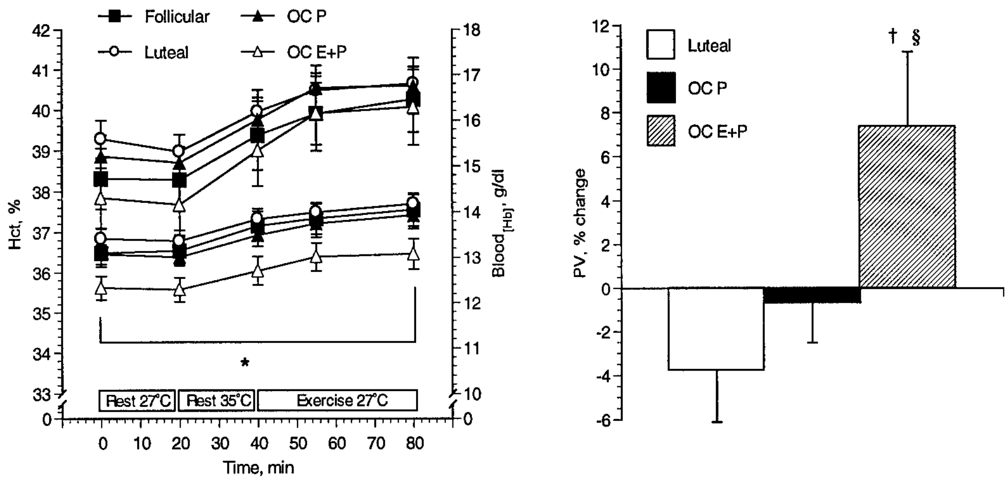

Figure 9. Plasma volume changes during exercise and percent changes at baseline relative to follicular phase. *Different from follicular phase. †Different from luteal phase. §Different from OC P.

Plasma osmolality and serum sodium concentrations were reduced before exercise during OC E+P relative to the follicular phase (Table 10, $P < 0.05$). Heart rate, stroke volume, cardiac output and blood pressure were unaffected by menstrual phase or oral contraceptive treatment prior to exercise (Table 11).

Passive heating. At the end of 20-min of passive heating, T_{es} during OC P was still greater relative to the follicular phase and OC E+P, but there were no differences between the menstrual phases. Plasma volume (Fig 9), plasma osmolality and sodium concentrations during OC E+P remained below the other trials during passive heating (data are not shown). Passive heating did not increase heart rate, cardiac output or blood pressure under any of the four conditions (Table 11).

Exercise responses. Exercise increased T_{es} during all four trials, but increased the greatest during OC P (Fig. 10, $P < 0.05$), and T_{sk} changed little during exercise. (Fig 11) Exercise SR was similar across all trials (Fig 12), but the T_{es} threshold for sweating onset was greater during the luteal phase and OC E+P relative to the follicular phase (Fig. 13, Table 12, $P < 0.05$). The changes in Hct and [Hb] (Fig 9) demonstrate that PV changes during exercise were similar across all trials. As with the other time periods, P_{Osm} and $S_{[Na+]}$ were reduced during OC E+P relative to the other trials (data not shown). Heart rate, stroke volume, cardiac out and blood pressure increased similarly across trials during exercise (Table 11).

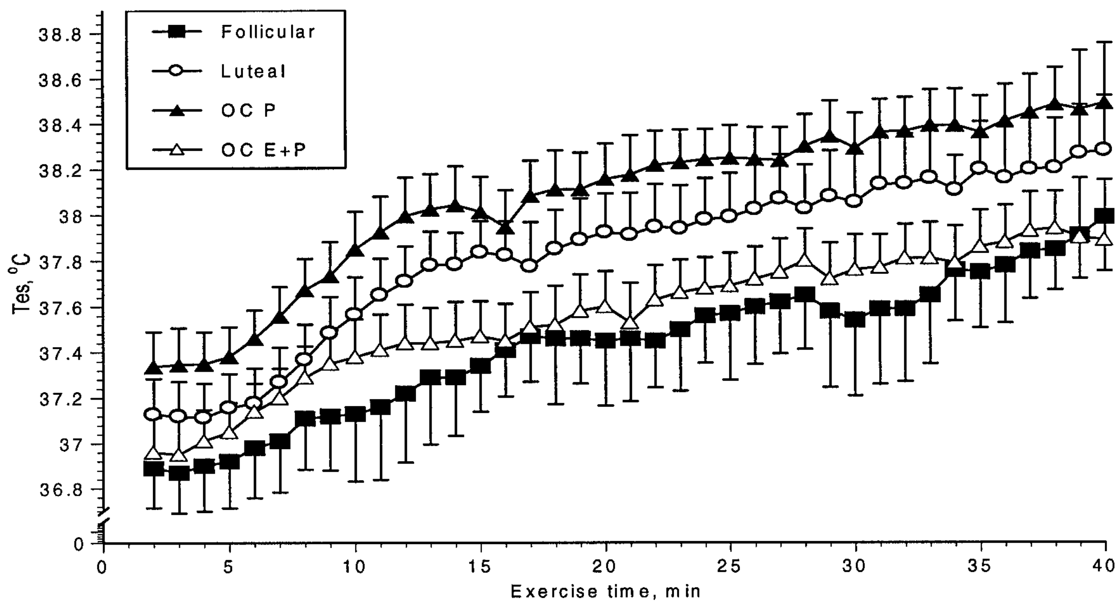

Figure 10. Esophageal (T_{es}) during 40-minutes of semirecumbent cycle exercise in the heat (35°C).

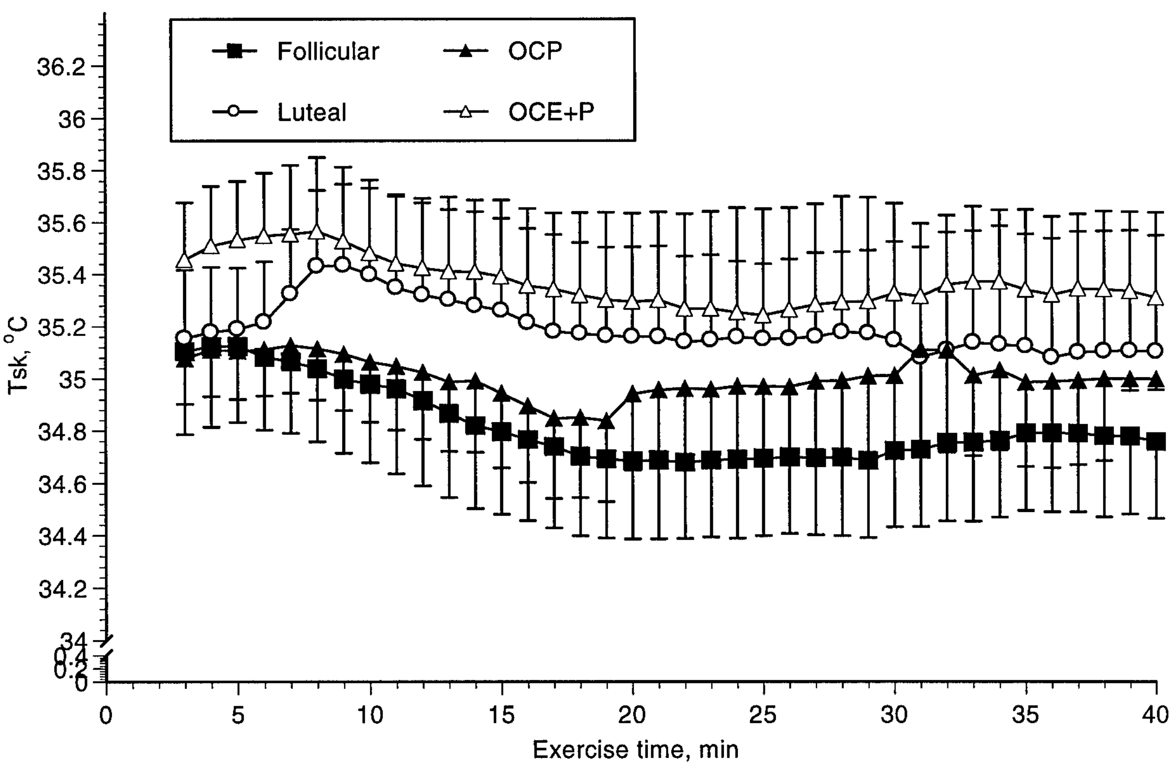

Figure 11. Weighted skin temperature (T_{sk}) during 40-minutes of semirecumbent cycle exercise in the heat (35°C).

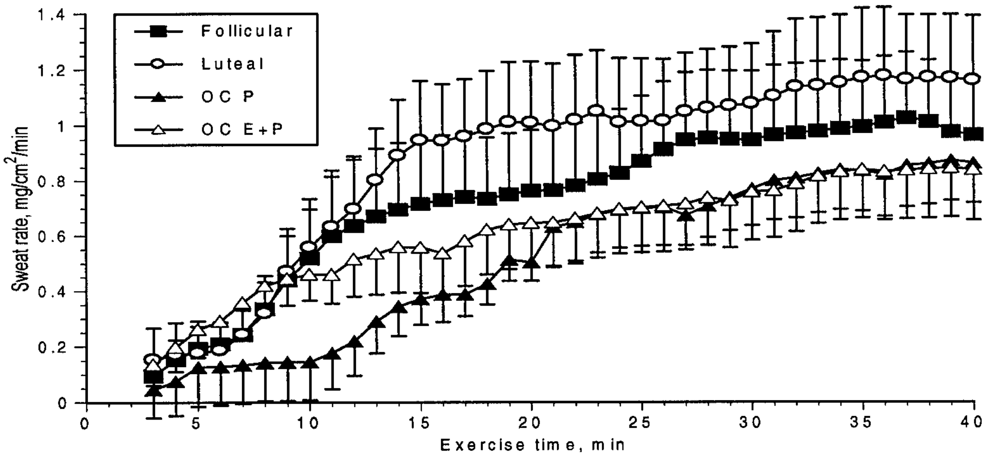

Figure 12. Arm sweat rate during exercise in the heat (35°C).

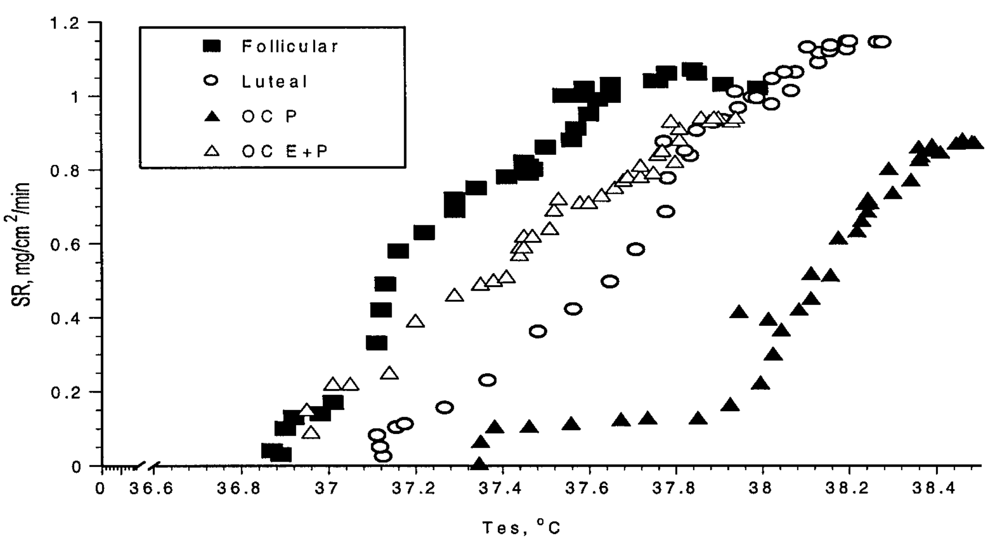

Figure 13. Arm sweat rate as a function of temperature changes during exercise at 35°C.

DISCUSSION

Our major findings are that unopposed progestin administration increased the regulated body temperature as both core temperature and the core temperature threshold for sweating increased, similar to what occurred during the mid-luteal phase of the menstrual cycle, and these thermoregulatory changes were blocked by treatment with estrogen plus progestin. This is the first report to address potential modulating effects of estrogen on the pronounced progesterone-related increase in regulated body temperature. In addition, ours is the first investigation in which a within-subject design that allowed sufficient time for tissue washout of synthetic estrogens and progestins between trials was used to determine the effects of synthetic estrogen and progestin on thermoregulation.

During OC E+P, the T_{es} threshold for sweating was ~0.5°C lower compared to the luteal phase indicating that synthetic estrogens and progestins do not elicit the same temperature responses as their endogenous counterparts. These differences may be due to differences in the direct or indirect actions of OCs on the CNS, to differences in the ratio or potency of the synthetic hormones, or to differential effects on peripheral influences on temperature regulation such as body fluid balance.

Charkoudian and Johnson (15) recently demonstrated that the core temperature threshold for active cutaneous vasodilation during passive heating was increased in women taking oral contraceptives containing estradiol [estrogen] and progestin compared to their responses after approximately 5 days of not taking the pill (15), a finding consistent with earlier findings of an increased core temperature threshold for initiation of cutaneous vasodilation during exercise in the luteal phase (39, 69). Postmenopausal women taking combined progestin and estrogen did not exhibit the same reduction in the T_{es} threshold for vasodilation or sweating seen in women taking only estrogen during exercise (12) suggesting that progestin blocks some of the estrogen-related thermoregulatory effects. However, Chang et al. (69) failed to find a change in core temperature following three days of estrogen administration to young women in their early follicular phase, perhaps because three days of estrogen administration may not be long enough to elicit temperature changes or that another hormone, such as FSH, facilitates hypothalamic neuronal adaptation to estradiol. Nonetheless, these reports indicate a disparity between chronic and acute effects of exogenous estrogens and progestins on temperature regulation.

Our data support earlier findings that estrogen with progestin administration does not alter the T_{es} threshold for thermoregulatory effector activation compared to the follicular phase (12). Moreover, we found that the estrogen component of the treatment reduced resting T_{es} by 0.58°C and the exercise T_{es} threshold for sweating by 0.68°C compared to progestin-only administration, indicating a profound modifying role for estrogen on the progesterone-induced core temperature increase. We suspect that the effects of these hormones occur via direct effects in the preoptic/anterior hypothalamus, the primary temperature-regulation area of the brain. Both estrogen and progesterone readily cross the blood-brain barrier, and may modulate thermoregulation via its action in the CNS, and sex steroid receptors have important effects on thermosensitive neurons in the brains of lower animals (52, 63). Progesterone inhibits warm-sensitive neuron activity, thus inhibiting heat loss mechanisms, and increasing body temperature (52). Conversely, estrogen inhibits cold and stimulates warms sensitive neurons Silva, 1986 #174], and should therefore inhibit heat-retaining mechanisms, excite heat loss mechanisms, and thus cause a decrease in the regulated body temperature.

To the extent that temperature is regulated by central mechanisms, estrogen and progesterone may exert their effects on the preoptic area and anterior hypothalamus by both transcription-dependent and transcription-independent mechanisms (22, 38). Various molecular mechanisms have been proposed to explain receptor-independent sex steroid action. Estrogens are metabolized to catechol estrogens, which, because of their structural similarity to catecholamines, may influence the metabolism and activity of these neurotransmitters in the hypothalamus (4, 6). In addition, A-ring reduced metabolites of progesterone influence the GABA-A-linked chloride ion channel, one of the most ubiquitous ion channels in the brain (45), thus allowing these ions to cross the membrane and regulate its excitability. However, extrapolation of basic mechanisms to predict physiological effects of estrogen and progesterone in humans has a number of limitations. These steroids and their metabolites have complex actions on different parts of the brain; indeed, a particular steroid may even have opposite effects on the same neurotransmitter system in different parts of the brain. This complexity makes it difficult to generalize findings from specific neuronal systems to other systems, or to predict how neuronal systems interact to regulate physiological systems, and to predict how the synthetic hormones found in oral contraceptives impact these receptors in humans.

Sex steroids may also act via a secondary mediator or pathway, such as cytokines (13) or heat shock proteins. However, these indirect mechanism are less likely to be involved in thermoregulatory changes during OC administration because neither IL-1β nor IL-6 are elevated during OC E+P administration (56) and estrogen administration had no effect on heat shock proteins in young women given estrogen; Chang, 1998 #782].

Our data together with earlier studies suggests that the effects on temperature regulation during sex hormone administration are more likely related to ratio of estrogen and progesterone, rather than the effects a single hormone. Endogenous progesterone and estrogen often have opposing effects on regulatory systems, and the presence of high levels of progesterones in the blood down-regulate estrogen receptors. This is consistent with the recent finding that the fall in the temperature thresholds for sweating onset of cutaneous vasodilation was related not only to the estrogen peak prior to ovulation, but also related to the ratio of E_2 to P_4 levels in the blood (68). The varying ratios of these hormones between the mid-luteal and OC E+P may explain the differing temperature responses in the present investigation. The ratio of progestin/estradiol *given* to each subject during OC E+P was 28.6, approximately 6-fold lower than the ratio of the progesterone/estradiol levels measured during the mid-luteal phase (151). However, we recognize that comparison of the different pill preparations is tenuous because the relative potency of synthetic estrogens and progestins found in oral contraceptives on the temperature regulation system is unknown. Furthermore, synthetic estrogens and progestins are metabolized at different rates among individual women so we cannot accurately predict the level of these hormones actually acting on tissue simply by knowing the quantity of the hormone administered.

While direct actions within the CNS may be a primary mechanism by which progesterone and estrogen exert their effects on the temperature regulation systems, the regulation of body temperature in humans is also interacts with systems that regulate volume and osmotic pressure of the extracellular fluid (48). Blood volume expansion improves the efficiency of cardiovascular and thermoregulatory responses during physical activity. When blood volume is expanded, cardiac stroke volume increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously, where heat transfer takes place. High estrogen levels in the blood are associated with plasma volume expansion (64, 70, 78), and plasma volume was at its lowest during the mid-luteal phase of the menstrual cycle coinciding with the highest core temperature and delayed sweating onset during exercise. However, although OC E+P was associated with a large increase in plasma volume compared to the follicular phase, there were no differences in the thermoregulatory responses during exercise. In contrast, the greater plasma volume during OC E+P compared to OC P may have played a role in their contrasting temperature responses. Indeed, HR and cardiac output was slightly reduced only during OC E+P compared to the other three trials.

We found that oral contraceptive pills containing block the thermoregulatory effects of oral contraceptive pills that contained only estrogen. These data extend earlier findings that estradiol may lower the thermoregulatory operating point, and also indicate that the synthetic hormones found in oral contraceptives do not elicit the same thermoregulatory responses as their endogenous counterparts. Differences in the ratio of the estrogen to progesterone or structural differences between the synthetic and endogenous hormones may be the cause of this disparity. Finally, plasma volume increased during exercise between the two oral contraceptive treatments, suggesting plasma volume expansion as a possible mechanism for the lower temperature during OC E+P treatment.

Responses to comments regarding the Progress Report from 1998:

The subjects are recruited without bias to ethnicity, and we make every attempt to include minorities in our subject pool. Based on recent census figures, minority groups comprise approximately 16 % of the population of Connecticut (8% Black, 6 % Hispanic, and 2 % Asian, and people of other races) and these figures are consistent with those reported from admissions at Yale-New Haven Hospital. Up to this point, 40 % of our subjects are African American or people from other minority ethnic origins, so we have been successful at recruiting a diverse group of subjects, which represent young, healthy women in the general population, although primarily they are from upper to upper-middle class, educated backgrounds.

The subjects for all three protocols are recruited through posted advertisements at Yale School of Medicine, School Yale School of Epidemiology (18) and Public Health and Yale University (undergraduates), and by word of mouth (1).

Protocol A:

For this protocol, we recruited a total of eleven subjects: one Hispanic, three African Americans and seven white subjects. One of the White subjects dropped out of the study for personal reasons, and another of the White subjects completed five of the six tests because the combined birth control pill induced extreme, chronic nausea. This left us with one Hispanic, three African American and five White subjects for our final analysis.

Protocol B:

For the second protocol, we recruited eleven subjects: two Hispanics, two Asian Americans, one Asian, three African Americans, and three white subjects. One Hispanic subject did not participate because of prior medical problems, one Black subject did not participate because of a positive Pap smear test, and two White and one Black subject were not used in the final analysis because they did not have normal menstrual cycles. This left us with one Hispanic, one African American, two white subjects and two Asian Americans and one Asian (Chinese).

Protocol C:

Up to this point, we have completed three subjects: one Hispanic, two White.

Two subjects who participated in Protocol A also participated in Protocol C, and one subject who participated in Protocol A also participated in Protocol B.

A small subset of the population can be used to extrapolate to the larger population in these instances because the measurements we make are precise and reproducible. We cannot however, extrapolate to make conclusions about men, or about other population of women, such as older women because aging has independent effects on the fluid regulatory and temperature regulatory systems.

We did see changes in dehydration and temperature responses to the oral contraceptive treatments, so one month is long enough to detect at least some important effects of the pills. In addition, there are cross-sectional studies comparing women on and off oral contraceptives on body water regulation (41-43, 56), that are consistent with our findings, suggesting that one month effects may be similar to chronic effects. However, our data cannot be extrapolated to indicate that the changes apparent after one month are similar to those during long-term oral contraceptive treatment.

Protocol C: Sex Hormone Effect on Osmotic Regulation of Thirst and Arginine Vasopressin

As discussed above, high estrogen states in women, such as occurs around the time of ovulation (82) and during pregnancy (21), are associated with water retention and plasma volume expansion. As we saw during dehydration (67) the mechanism for this estrogen-associated plasma volume expansion may involve alterations in the osmotic control of arginine vasopressin release and thirst.

Arginine vasopressin, the primary hormone involved in renal reabsorption of free water, is highly sensitive to changes in plasma tonicity. A strong and positive correlation (~ 0.95) exists between plasma osmolality and plasma concentration of arginine vasopressin. The slope of this relationship is used to assess osmotic control of arginine vasopressin release; a steeper slope is interpreted as heightened sensitivity of central osmoreceptors that cause the release of arginine vasopressin. When a body water deficit exists, arginine vasopressin also is sensitive to decreases in plasma volume through unloading of low-pressure baroreceptors (peripheral mechanism). The sense of thirst is also sensitive to elevated plasma tonicity and decreased plasma volume, leading to increased fluid intake when water is available. Therefore, under conditions of high plasma tonicity and body water deficit, restoration of body water is achieved through a combination of thirst-induced drinking and vasopressin-mediated renal water retention.

Plasma arginine vasopressin concentration is elevated in the presence of a high concentration of plasma estrogens in humans (23, 24). Plasma concentration of arginine vasopressin is increased during the mid-follicular phase of the menstrual cycle in young women (23) and following the administration of exogenous estrogen in post-menopausal women (24), although these increases are inconsistent when progesterone is increased along with estrogen ((23, 67). Pregnancy, a state characterized by elevated sex hormone levels, is associated with an altered threshold for arginine vasopressin release due to normal AVP levels combined with reduced P_{Osm}. Using hypertonic saline infusion in pregnant women, Davison et al. (21) noted a 6 mosmol/kg H_2O fall in the plasma osmolality threshold for arginine vasopressin release and a 10 mm fall (on a visual analogue scale) in the threshold for the onset of thirst, providing evidence for altered osmoregulatory control of arginine vasopressin and thirst responses in the presence of high plasma estrogen concentration.

It is likely that reproductive hormones act directly on the central nervous system to increase arginine vasopressin release. Estrogen can cross the blood brain barrier and gain access to hypothalamic sites (the paraventricular and supraoptic nucleii) that control arginine vasopressin release and some neurons in these sites bind estrogen (57, 63). In addition, estrogen may affect arginine vasopressin release indirectly through catecholaminergic and/or pro-opio melanocortin-immunopositive neurons which bind estrogen and project to the paraventricular and supraoptic nucleii (35), or by altering norepinephrine turnover (20).

This final protocol addressing female sex hormone effects on body fluid regulation was designed to explore the hypothesis that the mechanism for the expanded plasma volume associated with estrogen administration is increased responsiveness of thirst and arginine vasopressin to osmotic stimuli, resulting in increased fluid intake and renal water retention. We will relate the changes in thirst and arginine vasopressin to their expected responses; i.e. alterations in fluid intake and the renal reabsorption of free water.

METHODS

Study design:

As of this date, we have finished testing on three subjects, so only their data will be reported here. Two other subjects have finished one and three of the experimental days, respectively. Two other subjects will join the study within the next two to three weeks. We will continue our recruiting efforts to include a total of 10 subjects in this protocol.

Subjects are healthy, nonsmoking women (age 30 ± 3 y, range 25-34 y), with no contraindications to oral contraceptive use. All subjects are interviewed about their medical history, have medical and gynecological examinations and provided written confirmation of a negative Papanicolaou smear within one year of being admitted to the study. Each woman participates in four experiments, two baseline hypertonic saline infusion tests (one in the follicular and one in the luteal phase of the menstrual cycle), and one hypertonic saline infusion test while taking each type of oral contraceptive (two total). Estrogen and progesterone vary across the menstrual cycle, so the study design employs a hypertonic saline infusion test conducted in the early-follicular phase, 2-4 days after the beginning of menstrual bleeding (low estrogen and progesterone), one for each pill treatment, and one conducted in the mid-luteal phase, 7-9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel Corp, San Diego, CA). If the subject does not ovulate during a given cycle, she repeats the ovulation prediction test during the next cycle. Following two consecutive non-ovulatory cycles, the subject is excluded from further study. To verify phase of the menstrual cycle, plasma levels of estrogen and progesterone were assessed from the pre-exercise blood sample prior to undertaking the infusion protocol.

After completing the baseline hypertonic saline infusion tests, the subjects again undergo hypertonic saline infusions after four weeks of either continuous combined (estrogen-progestin, OC E+P) or progestin-only (OC P) oral contraceptive treatment (random assignment). Following a 4-week "washout" period, the subjects cross-over to the other pill treatment.

During OC E+P, subjects receive 0.035 mg of ethinyl estradiol and 1 mg of the progestin, norethindrone daily. During OC P treatment, subjects received 1 mg/day of the progestin, norethindrone.

Infusion studies

For each experiment, the subjects arrive at the laboratory at approximately 9:00 am, after eating a light (~ 300 kcal) breakfast. Upon reporting to the laboratory the subject voids her bladder, enters an environmental chamber (27°C, 30 % rh), is weighed to the nearest 10 g on a beam balance, and rests seated for a 60 min control period. During this period, a 20-ga teflon catheter is placed in an antecubital or forearm vein in each arm and baseline arterial blood pressure and heart rate recorded. Catheter patency is maintained by a heparin block (20 U/ml). At the end of the 60-min, a control blood sample is taken, thirst perception assessed and a urine sample collected. Following these control samples, hypertonic (3.0% NaCl) saline will be infused at a rate of $0.1\ ml \cdot min^{-1} \cdot kg^{-1}$ BW for 120 min into one of the catheters. Blood is sampled at 10, 20, 30, 40, 50, 60, 75, 105, and 120 min during the infusion from the other catheter, and a urine sample is obtained at the end of infusion. Following a 30 minute seated recovery period, the subject drinks water (15 ml/kg BW) over the next 30 minutes. Thirty minutes after drinking is complete, plasma volume is determined with Evan's Blue dye procedure (see below). Blood samples are obtained at 30, 60, and 120 min following infusion. Urine is collected at 60 and 120 min of drinking. Thirst perception is assessed at all blood sampling times. The subjects are weighed at the end of the infusion period, after the drinking period, and again at the end of the protocol.

All blood samples are analyzed for hematocrit, hemoglobin, total protein, osmolality, and the concentrations of creatinine, glucose, urea nitrogen, sodium, potassium, and arginine vasopressin. The control blood sample is also be analyzed for 17-β-estradiol and progesterone. Blood samples at control, at the end of the infusion, and at 60 and 120 min following the infusion will be analyzed for the concentration of atrial natriuretic peptide, aldosterone, and plasma renin activity. Urine will be analyzed for volume, osmolality, sodium, potassium, and creatinine concentrations

Blood sampling

All blood sampling is done via a 20 gauge Intracath catheter placed in an arm vein. Subjects are semi-recumbent during placement of the catheter and are seated for 60 min prior to sampling to ensure a steady state in plasma volume and constituents. Blood samples were separated immediately into aliquots. The first was analyzed for hemoglobin and hematocrit. A second aliquot was transferred to a heparinized tube, and a third aliquot for the determination of serum sodium and potassium concentrations was placed into a tube without anticoagulant. All other aliquots were placed in tubes containing EDTA. The tubes were centrifuged and the plasma taken off the heparinized sample analyzed for sodium, potassium, osmolality, glucose, urea creatinine and aldosterone. The EDTA samples were analyzed for concentrations of arginine vasopressin and atrial natriuretic peptide and plasma renin activity.

Blood volume

Absolute blood volume is measured by dilution of a known amount of Evan's blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing has occurred (10, 20 and 30 min). Plasma volume was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, taking into account 1.5% lost from the circulation within the 10 min. Blood volume is calculated from plasma volume and hematocrit concentration corrected for peripheral sampling.

Thirst ratings

The perception of thirst is assessed by asking the subject to make a mark on a line rating scale in response to the question, 'How thirsty do you feel now?'. The line is 175 mm in length and is marked 'not at all' on one end and 'extremely thirsty' at the 125 mm point. We tell subjects that they can mark beyond the 'extremely thirsty' point if they wish and may even extend the line if they feel it necessary. This method was developed by Marks et al. (46) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and plasma osmolality during hypertonic saline infusion and dehydration in young volunteers.

Calculations

Changes in plasma volume are estimated from changes in hemoglobin (Hb) and hematocrit (Hct) concentrations from the control (pre-exercise) sample according to the equation:

$$\% \Delta PV = 100 [[(Hb_b)/(Hb_a)][(1-hct_a \cdot 10^{-2})]/[(1-hct_b \cdot 10^{-2})]] - 100$$

where subscripts a and b denote measurements at time a and control, respectively. Hemoglobin is measured in triplicate by the cyanomethemoglobin technique and hematocrit in triplicate by the microhematocrit method.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na+}) are calculated from the following equations:

$$FE_{H_2O} = (U_V/GFR) \cdot 100$$

$$FE_{Na+} = (U_V \cdot [Na^+]_u/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \cdot [Na^+]_s$$

where the subscripts f and u are glomerular filtrate and urine respectively, U_v is urine flow rate, and $[Na^+]_s$ is $[Na^+]_s$ in protein-free solution (mEq/kg H_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Blood analysis:

Plasma and urine sodium and potassium are measured by flame photometry (Instrumentation Laboratory model 943), plasma osmolality by freezing point depression (Advanced Instruments 3DII), and plasma proteins by refractometry. Plasma glucose, urea and creatinine concentrations are determined by colorimetric assay (Sigma Diagnostic Products). Plasma renin activity, plasma concentrations of aldosterone, atrial natriuretic peptide, arginine vasopressin, 17-β-estradiol and progesterone are measured by radioimmunoassay. The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

Data analysis. For each subject, osmotic regulation of arginine vasopressin and thirst are determined by plotting plasma concentration of arginine vasopressin and thirst as functions of plasma osmolality during hypertonic saline infusion. The sensitivity of thirst and arginine vasopressin to changes in plasma osmolality provides the slope of this relationship, and the intercept provides the threshold for thirst onset and arginine vasopressin release. Body water handling is determined through the assessment of overall fluid balance and the renal clearance of free water, osmols and sodium. Plasma concentrations of the fluid and sodium-regulating hormones, aldosterone, atrial natriuretic peptide and plasma renin activity are assessed to determine the mechanisms by which renal water and osmoregulatory function are altered by sex hormone administration or suppression.

Statistics. The variables over time (control tests, hormone intervention tests) will be analyzed by conditions (combined estrogen, progestin vs. progestin-only) ANOVA for repeated measures. When significant differences are found, post hoc testing will be applied to determine differences between means. Differences will be considered statistically significant when $P<0.05$.

Sample size calculation. The primary variables used to determine changes in body water regulation, the osmotic regulation of arginine vasopressin are plasma concentrations of arginine vasopressin and thirst. Expected plasma arginine vasopressin and thirst responses within and between groups are derived from data from our laboratory using subjects of the same age (26, 66). In an earlier study, during hypertonic saline infusion, plasma arginine vasopressin increased by 5.29 pg/ml, and thirst perception by 88 mm on a visual anaolog scale. An estimate of the pooled standard deviation for the group was 1.81 pg/ml and 23 mm, for arginine vasopressin and thirst respectively.

The desired statistical test is two-sided at the 5% significance level, with 80% power to detect a difference. Based on our previous work, 80% power is sufficient to detect a significant alteration in plasma arginine vasopressin concentration and fluid balance. For a two-sided test, $Z_{(a)} = 1.96$, and for 80% power, $Z_{(\beta)} = 0.84$. The formula for calculating sample size for continuous response variables is (18):

$$N = 2[(Z_{(a)} + Z_{(\beta)})^2 (s)^2 /(d)^2]$$

Substituting the values the sample size is 8 subjects per group.

PRELIMINARY RESULTS
(n=3).

The data on the three complete subjects is presented in Table 14.

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APPENDIX A

Tables 1-13

	Follicular	**Luteal**	**OC E+P**	**Follicular**	**Luteal**	**OC P**
BW, kg	61.4 ± 4.1	61.8 ± 4.1	61.6 ± 3.8	60.7 ± 3.7	61.1 ± 3.4	60.0 ± 3.5
$P_{[E_2]}$, pg/ml (range)	27.3 ± 5.6 (12.3-40.8)	105.1 ± 26.2 (63.6-189.6)	> 12.0	26.1 ± 6.7 (13.1-36.2)	146.7 ± 38.3 (61.1-222.0)	25.1 ± 5.3 (6.4-26.7)
$P_{[P_4]}$, ng/ml (range)	1.3 ± 0.6 (0.3-2.2)	8.7 ± 3.1 (5.2-19.1)	> 0.02	0.49 ± 1.0 (0.4-0.8)	9.8 ± 2.2 (5.2-18.3)	> 0.02
P_{Osm}-$P_{[AVP]}$ slope $(pg \cdot ml^{-1}) \cdot mOsm^{-1}$	0.47 ± 0.11	0.51 ± 0.18	0.49 ± 0.12	0.49 ± 0.14	0.55 ± 0.17	0.46 ± 0.14
P_{Osm}-$P_{[AVP]}$ *x*-intercept, mOsm/kg H_2O	282 ± 1	$278 \pm 1^{*}$	$276 \pm 2^{\#}$	283 ± 1	$279 \pm 1^{*}$	280 ± 2
P_{Osm}-thirst slope, mm/mOsm	13.7 ± 3.5	14.0 ± 2.7	13.3 ± 3.7	12.8 ± 1.7	12.9 ± 2.9	13.7 ± 2.1
P_{Osm}-thirst *x*-intercept, mm	280 ± 3	278 ± 2	276 ± 2	280 ± 1	279 ± 2	280 ± 2

Table 1. Subject characteristics.

Table 2. Blood responses at rest, during dehydration and *ad libitum* drinking.

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
$S[Na^+]$, mEq/l						
Follicular	$137.9 \pm 0.5^{*\#}$	$141.3 \pm 0.9^{*\#}$	$140.5 \pm 0.9^{\#}$	$137.1 \pm 0.5^{*\#}$	$136.2 \pm 0.5^{*\#}$	$136.1 \pm 0.5^{*\#}$
Luteal	136.7 ± 0.6	139.6 ± 0.9	139.2 ± 0.8	136.2 ± 0.7	135.3 ± 0.5	134.9 ± 0.4
OC E + P	136.2 ± 0.6	139.9 ± 0.7	138.9 ± 0.7	135.9 ± 0.4	135.7 ± 0.4	134.8 ± 0.7
$S[K^+]$, mEq/l						
Follicular	3.85 ± 0.10	4.75 ± 0.11	4.18 ± 0.06	4.31 ± 0.05	4.19 ± 0.08	4.03 ± 0.08
Luteal	3.90 ± 0.10	4.77 ± 0.08	4.18 ± 0.07	4.35 ± 0.01	4.28 ± 0.08	4.14 ± 0.08
OC E + P	4.03 ± 0.10	4.82 ± 0.10	4.31 ± 0.07	4.42 ± 0.12	4.29 ± 0.0.7	4.17 ± 0.06
TP, g/l						
Follicular	6.7 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Luteal	6.8 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
OC E + P	6.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
$S[Na^+]$, mEq/l						
Follicular	$137.7 \pm 0.4^{*\S}$	$141.3 \pm 0.9^{*\S}$	$140.4 \pm 0.6^{*\S}$	$137.2 \pm 0.8^{*\S}$	$136.8 \pm 0.6^{*\S}$	$136.3 \pm 0.5^{*\S}$
Luteal	136.8 ± 0.4	140.2 ± 0.9	139.1 ± 0.8	136.2 ± 0.6	135.5 ± 0.7	134.7 ± 0.5
OC P	136.6 ± 0.5	140.6 ± 1.5	139.4 ± 0.8	136.5 ± 0.7	136.5 ± 0.8	136.0 ± 0.6
$S[K^+]$, mEq/l						
Follicular	3.86 ± 0.08	4.68 ± 0.11	4.14 ± 0.08	4.21 ± 0.07	4.09 ± 0.06	3.98 ± 0.04
Luteal	3.97 ± 0.08	4.94 ± 0.13	4.25 ± 0.06	4.41 ± 0.05	4.31 ± 0.07	4.02 ± 0.06
OC P	3.87 ± 0.11	4.70 ± 0.15	4.26 ± 0.13	4.12 ± 0.14	4.05 ± 0.08	3.90 ± 0.07
TP, g/l						
Follicular	6.7 ± 0.1	7.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.5 ± 0.1
Luteal	6.9 ± 0.1	7.5 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
OC P	6.8 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

Table 3. Blood responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Hct , %						
Follicular	36.3 ± 0.8*#	38.1 ± 0.9‡	36.6 ± 0.7	36.3 ± 0.8*#	36.3 ± 0.7*#	36.2 ± 0.8*#
Luteal	36.8 ± 1.0†	39.7 ± 0.8	37.7 ± 0.9†	37.1 ± 0.9†	37.0 ± 0.9†	37.0 ± 1.0†
OC E + P	35.7 ± 0.6δ	38.0 ± 0.8δ	36.4 ± 0.9δ	35.8 ± 0.9δ	35.6 ± 0.8δ	35.2 ± 0.7δ
Hb, g/dl						
Follicular	12.2 ± 0.2	13.0 ± 0.3	12.5 ± 0.3	12.1 ± 0.3	12.1 ± 0.3	12.1 ± 0.3
Luteal	12.5 ± 0.4	13.5 ± 0.4	12.8 ± 0.4	12.4 ± 0.4	12.4 ± 0.4	12.4 ± 0.4
OC E + P	11.9 ± 0.3δ	12.8 ± 0.3δ	12.1 ± 0.2δ	11.9 ± 0.2δ	11.7 ± 0.2δ	11.7 ± 0.2δ
PV, % change						
Follicular	---	-8.6 ± 1.3	-2.6 ± 1.6	1.3 ± 1.6	1.2 ± 1.7	2.5 ± 1.8
Luteal	---	-9.5 ± 2.6	-3.3 ± 2.0	0.2 ± 1.4	0.7 ± 1.6	0.5 ± 1.5
OC E + P	---	-7.9 ± 1.2	-0.5 ± 1.2	1.9 ± 1.3	3.6 ± 1.0	5.1 ± 1.7
$P_{[AVP]}$, pg/ml						
Follicular	1.3 ± 0.2	4.0 ± 0.8	3.3 ± 0.9	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3
Luteal	1.2 ± 0.2	3.8 ± 0.7	3.0 ± 0.7	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.4
OC E + P	1.6 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	2.7 ± 0.7	1.9 ± 0.4	2.3 ± 0.4
TP, g/l						
Follicular	6.7 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Luteal	6.8 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
OC E + P	6.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Hct , %						
Follicular	36.4 ± 0.7	37.5± 0.8	36.2 ± 0.7	35.8 ± 0.5	35.8 ± 0.5	35.4 ± 0.6
Luteal	37.9 ± 0.9††	40.0 ± 1.2††	38.4 ± 1.0††	37.8 ± 0.9††	37.9 ± 1.1††	37.6 ± 1.0††
OC P	37.1 ± 0.9	39.0 ± 0.9	37.2 ± 1.1	36.1 ± 0.9	36.3 ± 1.0	36.4 ± 0.8
Hb, g/dl						
Follicular	12.3 ± 0.4	13.2 ± 0.5	12.4 ± 0.4	12.1 ± 0.4	12.1 ± 0.4	11.9 ± 0.4
Luteal	13.0 ± 0.4	13.7 ± 0.5	12.9 ± 0.4	12.6 ± 0.4	12.6 ± 0.4	12.6 ± 0.4
OC P	12.6 ± 0.4	13.3 ± 0.4	12.5 ± 0.4	12.2 ± 0.3	12.2 ± 0.4	12.4 ± 0.4
PV, % change						
Follicular	---	-7.5 ± 1.2	0.0 ± 1.4	2.3 ± 1.1	3.1 ± 1.1	5.0 ± 0.7
Luteal	---	-7.4 ± 1.0	0.1 ± 1.1	3.2 ± 0.1	0.8 ± 1.2	1.6 ± 1.1
OC P	---	-6.5 ± 1.0	0.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.3	5.2 ± 1.6
$P_{[AVP]}$, pg/ml						
Follicular	1.2 ± 0.4	3.7 ± 1.0	2.5 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	1.6 ± 0.4
Luteal	1.1 ± 0.3	4.8 ± 1.4	2.3 ± 0.6	2.0 ± 0.5	1.9 ± 0.6	1.9 ± 0.6
OC P	1.0 ± 0.2	4.0 ± 1.2	2.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.5 ± 0.4
TP, g/l						
Follicular	6.7 ± 0.1	7.3 ± 0.2	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.1	6.5 ± 0.1
Luteal	6.9 ± 0.1	7.5 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
OC P	6.8 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.2

	Pre-exercise	Exercise	Rehydration			
	0 min	150 min	0 min	60 min	120 min	180 min
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	100 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC E + P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	100 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6

Table 4. Thirst responses to dehydrating exercise.

Table 5. Renal osmoregulatory responses at rest, and during dehydration and *ad libitum* drinking.

	Pre-Exercise	End-exercise	Rehydration		
	0 min	150 min	60 min	120 min	180 min
U_v ml/min					
Follicular	3.6 ± 1.1	1.1 ± 0.2	0.7 ± 0.1	2.0 ± 0.6	2.7 ± 0.8
Luteal	4.4 ± 0.9	1.5 ± 0.2	0.5 ± 0.1	1.1 ± 0.4	1.7 ± 0.5
OC P + E	3.3 ± 0.7	0.9 ± 0.2	0.5 ± 0.0	0.6 ± 0.1	0.9 ± 0.3
U_{Osm}, mosmol/kg H_2O					
Follicular	290 ± 123	509 ± 79	790 ± 95	577 ± 135	451 ± 152
Luteal	148 ± 29	339 ± 57	833 ± 52	633 ± 125	481± 124
OC P + E	274 ± 97	502 ± 86	889 ± 48	792 ± 94	675 ± 120
U_{Osm}/P_{Osm}					
Follicular	1.0 ± 0.4	1.8 ± 0.3	3.0 ± 0.3	2.2 ± 0.5	1.8 ± 0.6
Luteal	0.5 ± 0.1	1.1 ± 0.2	3.0 ± 0.2	1.9 ± 0.5	1.9 ± 0.5
OC P + E	1.1 ± 0.4	1.7 ± 0.3	3.2 ± 0.2	2.5 ± 0.5	2.3 ± 0.5
C_{H_2O}, ml/min					
Follicular	1.7 ± 1.1	-0.5 ± 0.2	-1.0 ± 0.2	-0.4 ± 0.3	1.0 ± 0.6
Luteal	2.5 ± 0.8	0.0 ± 0.3	-1.0 ± 0.1	-0.5 ± 0.3	0.1 ± 0.5
OC P + E	1.5 ± 0.7	-0.4 ± 0.2	-1.0 ± 0.1	-1.0 ± 0.1	-0.6 ± 0.2
C_{Osm}					
Follicular	1.9 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
Luteal	1.8 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
OC P + E	1.7 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1

	Pre-Exercise	End-exercise	Rehydration		
	0 min	150 min	60 min	120 min	180 min
U_v ml/min					
Follicular	5.0 ± 1.2	1.3 ± 0.3	0.6 ± 0.1	1.3 ± 0.4	1.7 ± 0.5
Luteal	4.6 ± 0.8	3.5 ± 0.5	0.9 ± 0.1	1.4 ± 0.5	1.7 ± 0.5
OC P + E	4.4 ± 0.6	3.7 ± 0.7	0.8 ± 0.7	1.5 ± 0.5	2.0 ± 0.5
U_{Osm},, mosmol/kg					
H_2O	171 ± 48	410 ± 81	876 ± 77	662 ± 132	486 ± 128
Follicular	166 ± 43	406 ± 77	837 ± 55	635 ± 137	567 ± 139
Luteal	125 ± 23	387 ± 58	799 ± 39	553 ± 130	415 ± 109
OC P					
U_{Osm},/P_{Osm},					
Follicular	0.6 ± 0.2	1.4 ± 0.3	2.7 ± 0.5	2.6 ± 0.5	1.8 ± 0.5
Luteal	0.6 ± 0.2	1.4 ± 0.3	3.0 ± 0.2	2.2 ± 0.5	1.9 ± 0.5
OC P	0.4 ± 0.1	1.4 ± 0.2	2.8 ± 0.2	1.6 ±0.5	1.6 ± 0.4
C_{H_2O}, ml/min					
Follicular	3.0 ± 1.0	-0.1 ± 0.2	-1.1 ± 0.1	-0.5 ± 0.5	0.1 ± 0.5
Luteal	2.4 ± 0.9	-0.2 ± 0.2	-1.0 ± 0.1	-0.2 ± 0.4	0.1± 0.6
OC P	2.6 ± 0.6	-0.4 ± 0.2	-0.9 ± 0.1	0.1 ± 0.5	0.4 ± 0.5
C_{Osm}					
Follicular	2.0 ± 0.3	1.4 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
Luteal	2.0 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
OC P	1.8 ± 0.2	1.6 ± 0.3	1.4 ± 0.2	1.5 ± 0.1	1.5 ± 0.3

	Pre-Exercise	End-exercise		Rehydration	
	0 min	150 min	60 min	120 min	180 min
GFR, ml/min					
Follicular	113 ± 8	83 ± 10	74 ± 10	92 ± 12	83 ± 9
Luteal	119± 5	94 ± 7	72 ± 9	84 ± 8	91 ± 11
OC E + P	111 ± 6	89 ± 13	86 ± 9	88 ± 12	91 ± 9
FE_{Na+}, %					
Follicular	0.49 ± 0.09	0.66 ± 0.16	0.93 ± 0.14	0.60 ± 0.05	0.54 ± 0.05
Luteal	0.32 ± 0.06	0.37 ± 0.09	0.65 ± 0.14	0.51 ± 0.06	0.49 ± 0.08
OC E + P	0.36 ± 0.07	0.42 ± 0.11	0.66 ± 0.10	0.62 ± 0.12	0.78 ± 0.31
U_{Na+}, mEq					
Follicular	5.2 ± 0.8	13.4 ± 2.8$^{*\#}$	9.3 ± 1.3	5.7 ± 0.9	4.0 ± 0.4
Luteal	3.3 ± 0.6	7.2 ± 1.5	6.0 ± 0.8	5.0 ± 1.5	4.8 ± 1.5
OC E + P	3.6 ± 0.6	7.4 ± 1.6	6.7 ± 0.8	4.3 ± 0.6	4.2 ± 0.9
U_{K+}, mEq					
Follicular	2.2 ± 0.4	10.30 ± 2.1	7.2 ± 1.4	5.2 ± 1.4	3.3 ± 0.4
Luteal	4.1 ± 1.9	11.5 ± 2.0	5.2 ± 1.2	3.4 ± 0.9	2.8 ± 0.6
OC E + P	1.9 ± 0.4	8.7 ± 1.6	5.8± 1.1	4.1± 1.1	4.1± 0.8
$[Na^+]_u/[K^+]_u$					
Follicular	2.2 ± 0.2	1.0 ± 0.2	2.5 ± 0.8	4.2 ± 2.1	1.3 ± 0.3
Luteal	1.8 ± 0.6	0.7 ± 0.2	2.4 ± 0.9	6.5 ± 4.9	1.7 ± 0.4
OC E + P	2.5 ± 0.8	0.8 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	1.1 ± 0.2

	Pre-Exercise	End-exercise		Rehydration	
	0 min	150 min	60 min	120 min	180 min
GFR, ml/min					
Follicular	119 ± 9	89 ± 9	82 ± 9	85 ± 5	96 ± 4
Luteal	115 ± 8	96 ± 5	93 ± 8	111 ± 9	103 ± 9
OC P	120 ± 8	87 ± 7	79 ± 7	87 ± 6	90 ± 6
FE_{Na+}, %					
Follicular	0.36 ± 0.09	0.43 ± 0.10	0.71 ± 0.14	0.57 ± 0.11	0.46 ± 0.12
Luteal	0.35 ± 0.05	0.42 ± 0.12	0.55 ± 0.19	0.35 ± 0.07	0.33 ± 0.05
OC P	0.35 ± 0.05	0.47 ± 0.14	0.58 ± 0.10	0.44 ± 0.08	0.41 ± 0.09
U_{Na+}, mEq					
Follicular	4.5 ± 1.1	10.7 ± 2.5*§	7.6 ± 1.9	6.7 ± 1.6	4.8 ± 1.0
Luteal	4.3 ± 0.7	8.7 ± 1.8	5.9 ± 1.3	3.5 ± 0.6	3.3 ± 0.5
OC P	3.6 ± 0.8	8.5 ± 3.3	6.1 ± 1.3	3.1 ± 0.7	2.8 ± 0.6
U_{K+}, mEq					
Follicular	2.1 ± 0.7	8.0 ± 1.7	6.2 ± 1.6	4.0 ± 1.2	2.6 ± 0.4
Luteal	2.1 ± 0.5	11.2 ± 1.4	5.5 ± 0.8	3.3 ± 0.3	3.2 ± 0.5
OC P	2.0 ± 0.5	8.8 ± 2.1	3.6 ± 0.4	2.5 ± 0.5	3.0 ± 0.6
$[Na^+]_u/[K^+]_u$					
Follicular	2.9 ± 0.9	1.3 ± 0.5	2.9 ± 1.2	2.2 ± 0.6	2.1 ± 0.4
Luteal	2.6 ± 0.8	0.9 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	1.0 ± 0.2
OC P	2.2 ± 0.8	0.9 ± 0.5	1.8 ± 0.7	1.7 ± 0.6	1.8 ± 0.6

Table 6. Renal electrolyte excretion at rest, during dehydration and *ad libitum* drinking.

	Exercise		Rehydration		
	Pre-	End-			
	0 min	150 min	0 min	120 min	180 min
HR, beats/min					
Follicular	77 ± 4	144 ± 6	86 ± 4	76 ± 3	75 ± 4
Luteal	75 ± 5	142 ± 5	88 ± 4	75 ± 6	80 ± 5
OC E+P	78 ± 3	135 ± 6	85 ± 6	77 ± 4	76 ± 4
MAP, mm Hg					
Follicular	83 ± 2	85 ± 3	77 ± 2	80 ± 2	79 ± 1
Luteal	82 ± 2	82 ± 3	76 ± 2	79 ± 2	78 ± 2
OC E+P	83 ± 1	84 ± 4	77 ± 2	77 ± 2	79 ± 1
SBP, mm Hg					
Follicular	113 ± 3	141 ± 7	110 ± 2	106 ± 2	108 ± 2
Luteal	115 ± 3	137 ± 6	109 ± 3	109 ± 4	109 ± 2
OC E+P	118 ± 2	145 ± 8	111 ± 2	109 ± 1	112 ± 1
DBP, mm Hg					
Follicular	69 ± 2	57 ± 2	61 ± 2	67 ± 3	64 ± 1
Luteal	66 ± 2	52 ± 3	60 ± 2	65 ± 3	62 ± 3
OC E+P	66 ± 1	54 ± 3	61 ± 2	61 ± 3	63 ± 2
PP, mm Hg					
Follicular	44 ± 3	84 ± 5	49 ± 2	39 ± 5	44 ± 2
Luteal	49 ± 5	83 ± 7	49 ± 4	44 ± 6	47 ± 4
OC E+P	52 ± 3	91 ± 6	50 ± 3	48 ± 3	49 ± 2

Table 7A. Cardiovascular responses to dehydration.

	Exercise		Rehydration		
	Pre	End			
	0 min	150 min	0 min	120 min	180 min
HR, beats/min					
Follicular	79 ± 3	145 ± 3	88 ± 4	73 ± 2	74 ± 4
Luteal	80 ± 4	142 ± 5	88 ± 4	75 ± 6	80 ± 5
OC P	81 ± 4	141 ± 7	92 ± 4	82 ± 4	81 ± 3
MAP, mm Hg					
Follicular	86 ± 2	82 ± 4	81 ± 4	78 ± 1	78 ± 2
Luteal	82 ± 2	84 ± 3	78 ± 3	79 ± 2	77 ± 2
OC P	81 ± 2	83 ± 3	78 ± 2	80 ± 2	79 ± 2
SBP, mm Hg					
Follicular	116 ± 2	140 ± 6	114 ± 4	109 ± 2	110 ± 2
Luteal	115 ± 3	137 ± 6	109 ± 3	109 ± 4	109 ± 2
OC P	116 ± 3	136 ± 4	110 ± 2	111 ± 2	110 ± 3
DBP, mm Hg					
Follicular	71 ± 3	53 ± 3	65 ± 4	62 ± 2	62 ± 3
Luteal	66 ± 2	58 ± 3	64 ± 5	63 ± 3	63 ± 2
OC P	64 ± 2	57 ± 4	62 ± 3	65 ± 2	64 ± 2
PP, mm Hg					
Follicular	45 ± 4	87 ± 5	48 ± 4	47 ± 3	49 ± 3
Luteal	42 ± 3	80 ± 5	42 ± 7	48 ± 3	43 ± 3
OC P	52 ± 4	79 ± 5	48 ± 3	47 ± 2	46 ± 3

Table 7B. Cardiovascular responses to dehydration.

	Follicular Phase		
	Pre-exercise	Exercise	Rehydration
	0 min	150 min	AUC‡
$P_{[ALD]}$, pg/ml			
Trial A	78 ± 12	275 ± 65	$228 \cdot 10^2 \pm 37 \cdot 10^2$
Trial B	96 ± 19	198 ± 47	$166 \cdot 10^2 \pm 30 \cdot 10^2$
PRA, $ng \cdot ml^{-1}$ $ANG \cdot hr^{-1}$			
Trial A	0.8 ± 0.2	3.9 ± 1.0	287 ± 60
Trial B	0.9 ± 0.2	3.4 ± 1.1	267 ± 62
$P_{[AVP]}$, pg/ml			
Trial A	1.3 ± 0.2	3.7 ± 0.8	399 ± 72
Trial B	1.2 ± 0.4	3.5 ± 0.8	374 ± 106
$P_{[ANP]}$, pg/ml			
Trial A	33.0 ± 3.9	88.1 ± 11.7	$78 \cdot 10^2 \pm 8 \cdot 10^2$
Trial B	38.0 ± 5.3	87.9 ± 12.1	$76 \cdot 10^2 \pm 8 \cdot 10^2$

	Luteal Phase		
	Pre-exercise	Exercise	Rehydration
	0 min	150 min	AUC‡
$P_{[ALD]}$, pg/ml			
Trial A	156.8 ± 21.8*	388.1 ± 43.1*	$330 \cdot 10^2 \pm 47 \cdot 10^2$*
Trial B	154.9 ± 20.6*	499.8 ± 51.0*	$460 \cdot 10^2 \pm 52 \cdot 10^2$*
PRA, $ng \cdot ml^{-1}$ $ANG \cdot hr^{-1}$			
Trial A	1.8 ± 0.4*	6.1 ± 1.7*	471 ± 113*
Trial B	1.7 ± 0.2*	4.2 ± 0.9*	653 ± 121*
$P_{[AVP]}$, pg/ml			
Trial A	1.2 ± 0.2	3.2 ± 0.6	347 ± 79
Trial B	1.1 ± 0.3	3.7 ± 1.1	496 ± 125
$P_{[ANP]}$, pg/ml			
Trial A	49.6 ± 5.6	109.2 ± 14.5	$94 \cdot 10^2 \pm 9 \cdot 10^2$
Trial B	54.6 ± 9.2	114.8 ± 22.2	$101 \cdot 10^2 \pm 14 \cdot 10^2$

Table 8. Fluid regulation hormones over two menstrual cycles.

	Cronbach's α	
	Follicular Phase	**Luteal Phase**
Resting $P_{[AVP]}$	0.49	0.25
Exercise $P_{[AVP]}$	$0.81^{\dagger}$	$0.98^{\dagger}$
Rehydration $P_{[AVP]}$	0.58	$0.96^{\dagger}$
$P_{[AVP]}$-P_{Osm} slope	$0.96^{\dagger}$	$0.81^{\dagger}$
$P_{[AVP]}$-P_{Osm} intercept	$0.90^{\dagger}$	$0.86^{\dagger}$
Resting $P_{[ANP]}$	$0.80^{\dagger}$	$0.80^{\dagger}$
Exercise $P_{[ANP]}$	$0.90^{\dagger}$	$0.87^{\dagger}$
Rehydration $P_{[ANP]}$	$0.93^{\dagger}$	$0.80^{\dagger}$
Resting PRA	0.49	0.51
Exercise PRA	0.72	$0.89^{\dagger}$
Rehydration PRA	0.67	$0.95^{\dagger}$
Resting $P_{[ALD]}$	0.55	0.66
Exercise $P_{[ALD]}$	0.66	$0.82^{\dagger}$
Rehydration $P_{[ALD]}$	0.64	0.76
Resting $P_{[E_2]}$	$0.85^{\dagger}$	$0.93^{\dagger}$
Resting $P_{[P_4]}$	0.62	$0.92^{\dagger}$

Table 9. Reliability of fluid regulation hormones over two menstrual cycles.

	Follicular	Luteal	OC P	OC E+P
BW, kg	53.8 ± 3.3	53.2 ± 3.0	53.3 ± 2.9	52.1 ± 3.1
$P[E_2]$, pg/ml	21 ± 6	104.5 ± 20.0	31.3 ± 10.0	10.0 ± 2.9
$P[P_4]$, ng/ml	0.7 ± 0.1	12.0 ± 1.8	0.6 ± 0.1	0.7 ± 0.1
Hct, %	38.3 ± 0.7	39.3 ± 0.5	38.9 ± 0.5	37.8 ± 0.7
[Hb], g/dl	13.1 ± 0.3	13.4 ± 0.2	13.1 ± 0.2	12.3 ± 0.3
P_{Osm}, mOsmol/kg	284 ± 1	283 ± 1	285 ± 1	282 ± 1*
$S_{[Na+]}$, mEq/l	137.8 ± 0.8	137.4 ± 0.5	138.0 ± 0.7	136.8 ± 0.9
$P[P_4]$/ $P[E_2]$	85.8 ± 51.6	151.3± 50.1		
Tes°C at 27°C	36.66 ± 0.21	37.11 ± 0.20*	37.61 ± 0.31*	37.03 ± 0.23
Tsk °C at 27°C	31.27 ± 0.38	31.66 ± 0.16	31.12 ± 0.22	31.82 ± 0.27
Tes °C at 35°C pre-exercise	36.98 ± 0.30	37.15 ± 0.32	37.54 ± 0.27*†	36.64 ± 0.13
Tsk °C at 35°C pre-exercise	35.08 ± 0.29	35.04 ± 0.14	35.21 ± 0.16	35.61 ± 0.24
Tes °C at 35°C 40-min of exercise	37.86 ± 0.16	38.31 ± 0.27	38.74 ± 0.35	37.75 ± 0.21
Tsk °C at 35°C 40-min of exercise	34.82 ± 0.26	34.99 ± 0.41	34.84 ± 0.24	35.01 ± 0.20

Table 10. Baseline subject characteristics.

	Rest 27°C	Rest 35°C	exercise 35°C 20 min	exercise 35°C 40 min
Heart rate, bpm				
Follicular	67 ± 3	69 ± 3	132 ± 8	140 ± 8
Luteal	67 ± 3	69 ± 4	127 ± 7	137 ± 7
OC P	66 ± 3	68 ± 3	131 ± 6	141 ± 7
OC E+P	64 ± 4	67 ± 4	126 ± 10	135 ± 9
Stroke volume, ml				
Follicular	81 ± 8	80 ± 7	98 ± 10	99 ± 10
Luteal	88 ± 8	88 ± 7	111 ± 11	111 ± 10
OC P	87± 6	87 ± 6	113 ± 10	110 ± 11
OC E+P	99 ± 7	95 ± 8	112 ± 8	115 ± 15
Cardiac output, l/min				
Follicular	5.3 ± 0.4	5.5 ± 0.4	12.9 ± 1.1	13.6 ± 1.2
Luteal	5.8 ± 0.5	6.0 ± 0.3	13.9 ± 1.2	14.9 ± 1.4
OC P	5.8 ± 0.4	6.0 ± 0.3	14.4 ± 0.9	15.2 ± 1.4
OC E+P	6.4 ± 0.5	6.2 ± 0.5	13.6 ± 1.0	15.1 ± 1.4
Mean arterial pressure, mm Hg				
Follicular	80 ± 4	81 ± 4	90 ± 4	92 ± 5
Luteal	80 ± 3	78 ± 3	92 ± 4	90 ± 5
OC P	78 ± 2	76 ± 2	88 ± 2	89 ± 2
OC E+P	77 ± 2	78 ± 2	93 ± 5	96 ± 5

Table 11. Cardiovascular responses during exercise in the heat.

	Follicular	Luteal	OC P	OC E+P
T_{es} threshold, °C	37.5 ± 0.2	$38.0 \pm 0.3^{*}$	$38.1 \pm 0.2^{*\dagger}$	37.5 ± 0.2
slope, $\Delta SR/\Delta T_{es}$	0.88 ± 0.28	1.08 ± 0.21	1.13 ± 0.30	0.86 ± 0.23
r^2	0.81 ± 0.05	0.90 ± 0.03	0.76 ± 0.05	0.87 ± 0.03

Table 12. Control of sweating during exercise in the heat.

	Follicular	Luteal	OC P	OC E+P
BW, kg	62.2 ± 4.6	60.6 ± 4.7	62.4 ± 5.2	61.8 ± 6.0
Hct, %	37.4 ± 0.8	38.6 ± 1.5	36.3 ± 1.2	36.0 ± 0.9
[Hb], g/dl	12.2 ± 0.4	12.6 ± 0.7	12.1 ± 0.5	12.2 ± 0.3
BV ml /kg BW	69.5 ± 1.3	63.7 ± 3.3	65.2 ± 2.3	67.8 ± 4.3
P_{Osm}, mOsmol/kg H_2O	287 ± 1	282 ± 2	282 ± 2	280 ± 2
$S_{[Na+]}$, mEq/l	139.4 ± 0.4	136.4 ± 0.6	138.4 ± 0.4	138.3 ± 0.6
P_{Osm}-thirst slope, mm/mOsm	6.1 ± 1.7	7.4 ± 0.4	5.1 ± 1.7	6.2 ± 1.7
P_{Osm}-thirst *x*-intercept, mOsm	286 ± 3	283 ± 3	280 ± 4	284 ± 3

Table 13. Subject characteristics and thirst responses during hypertonic saline infusion.

Text to tables

Table 1. Subject characteristics and responses to dehydration. Pre-exercise body weight (BW) and plasma concentrations of endogenous 17-β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OC E+P) and (progestin only, OC P) oral contraceptive pills. Slopes and abscissal intercepts of the individual subjects' plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{Osm}) and thirst-P_{Osm} relationships during dehydration in the early follicular and mid-luteal phases of the menstrual cycle and OC E+P and OC P. *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 2. Blood responses at rest, and during dehydration and *ad libitum* drinking. Serum concentrations of sodium ($S_{[Na+]}$) and potassium $S_{[K+]}$), and total protein concentration (TP). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P, §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 3. Blood responses at rest, and during dehydration and *ad libitum* drinking. Hematocrit (Hct), blood hemoglobin concentration (Hb), plasma volume (PV), plasma arginine vasopressin concentration ($P_{[AVP]}$) and total protein (TP).. *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P, †Difference between luteal phase and OC E+P. §Difference between follicular phase and OC P. ††Difference between luteal phase and OC P. δDifference between OC E+P and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 4. Thirst ratings (analog-rating scale) at rest, and during dehydration and *ad libitum* drinking.

Table 5. Renal osmoregulatory responses at rest, and during dehydration and *ad libitum* drinking. Urine flow (U_v), urine osmolality (U_{Osm}), plasma osmolality (P_{Osm}), free water clearance (C_{H_2O}), osmolar clearance (C_{Osm}). #Difference between follicular phase and OC E+P, †Difference between luteal phase and OC E+P. Data are expressed as mean ± SEM.

Table 6. Renal function and electrolyte excretion at rest, during dehydration and *ad libitum* drinking. Glomerular filtration rate (GFR), fractional excretion of sodium (FE_{Na+}), urine excretion of sodium (U_{Na+}) and potassium (U_{K+}), and ratio of urine sodium and potassium concentrations ($[Na^+]_u/[K^+]_u$). *Difference between the follicular and luteal phases. #Difference between follicular phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 7A. Cardiovascular responses to dehydration. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin oral contraception administration OC E+P, n=8). Data are expressed as mean ± SEM.

Table 7B. Cardiovascular responses to dehydration. Heart rate (HR), mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest and in response to 150 min dehydrating exercise and 180 of *ad libitum* rehydration in the follicular and luteal phases, and during progestin-only oral contraception administration (n=9). Data are expressed as mean ± SEM.

Table 8. Fluid regulation hormones over two menstrual cycles. Trial A and Trial B are the first and second trials within the specified menstrual phase. Plasma renin activity (PRA) and plasma concentrations of aldosterone ($P_{[ALD]}$), arginine vasopressin ($P_{[AVP]}$) and atrial natriuretic peptide ($P_{[ANP]}$) in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the early follicular and mid-luteal phases of the menstrual cycle. *Difference between the follicular and luteal phases. ‡Area under the curve (AUC, trapezoid). Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 9. Reliability of fluid regulation hormones over two menstrual cycles. Cronbach's α for reliability within two follicular and two luteal phase tests. †Cronbach's $\alpha \geq 0.80$ was considered reliable.

Table 10. Subject characteristics (27°C) and responses to passive heating (35°C) and exercise in the heat (35°C). Pre-exercise body weight (BW), plasma concentrations of endogenous 17-β estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$), hematocrit (Hct), blood hemoglobin concentration ([Hb]), plasma osmolality (P_{Osm}) and serum sodium concentration ($S_{[Na+]}$). Esophageal (Tes) and skin (Tsk) temperatures in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OC E+P) and (progestin only, OC P) oral contraceptive pills, and following 40-min of exercise at 35°C. *Difference from follicular. †Difference from OC E+P. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 11. Cardiovascular responses to passive heat and exercise. Heart rate (HR), stroke volume (SV), cardiac output (CO) mean (MAP), systolic (SBP), diastolic (DBP) and pulse (PP) blood pressures at rest (27°C) and in response to 20 min of passive heating (35°C) and 40 min of exercise (35°C) in the follicular and luteal menstrual phases. Data are expressed as mean ± SEM.

Table 12. Esophageal temperature for sweating during 40 min of exercise (35°C) in the early follicular and mid-luteal phases of the menstrual cycle and during administration of combined (estradiol + progestin, OC E+P) and (progestin only, OC P) oral contraceptive pills. *Difference from follicular. †Difference from OC E+P. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM.

Table 13. Subject characteristics and thirst responses during hypertonic saline infusion. Pre-infusion body weight (BW), blood volume (BV), hematocrit (Hct), blood hemoglobin

concentration ([Hb]), plasma osmolality (P_{Osm}) and serum sodium concentration ($S_{[Na+]}$), and (n=3).

Text to figures

Figure 1. Time line for sex hormone administration. (Figure embedded in text).

Figure 2. Plasma osmolality (P_{Osm}) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P, n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. $^{\#}$Difference between follicular phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at P <0.05. Data are expressed as mean ± SEM.

Figure 3. Plasma renin activity (PRA) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. $^{\#}$Difference between follicular phase and OC E+P. †Difference between luteal phase and OC E+P. Differences were accepted as significant at P <0.05. Data are expressed as mean ± SEM.

Figure 4. Plasma aldosterone concentration ($P_{[ALD]}$) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. †Difference between luteal phase and OC E+P. ††Difference between luteal phase and OC P. Differences were accepted as significant at P <0.05. Data are expressed as mean ± SEM.

Figure 5. Plasma atrial natriuretic peptide ($P_{[ANP]}$) at rest, and in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. †Difference between luteal phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at P <0.05. Data are expressed as mean ± SEM.

Figure 6. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in plasma osmolality (P_{Osm}) during dehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P, n=8) and progestin-only oral contraception administration (OC P, n=9). Data are expressed as mean ± SEM.

Figure 7. Cumulative renal sodium (Na^+) in response to dehydrating exercise and 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P n=8) and progestin-only oral contraception administration (OC P, n=9). *Difference between the follicular and luteal phases. $^{\#}$Difference between follicular phase and OC E+P. §Difference between follicular phase and OC P. Differences were accepted as significant at P <0.05. Data are expressed as mean ± SEM.

Figure 8. Body fluid balance after dehydrating exercise and during 180 min of *ad libitum* rehydration in the follicular and luteal phases, and during combined estradiol/progestin (OC E+P, n=8) and progestin-only oral contraception administration (OC P, n=9). #Difference between the follicular and OC E+P. †Difference between luteal phase and OC E+P. Differences were accepted as significant at $P < 0.05$. Data are expressed as mean ± SEM.

Figure 9. Plasma volume changes during exercise and % changes at baseline relative to follicular phase. *Different from follicular phase. †Different from luteal phase. § Different from OC P. . **(Figure embedded in text).**

Figure 10. Esophageal (T_{es}) during 40-minutes of semirecumbent cycle exercise in the heat (35°C). (Figure embedded in text).

Figure 11. Weighted skin temperature (T_{sk}) during 40-minutes of semirecumbent cycle exercise in the heat (35°C). (Figure embedded in text).

Figure 12. Arm sweat rate during exercise in the heat (35°C). (Figure embedded in text).

Figure 13. Arm sweat rate as a function of temperature changes during exercise at 35°C. (Figure embedded in text).

APPENDIX B
Figures 2-8

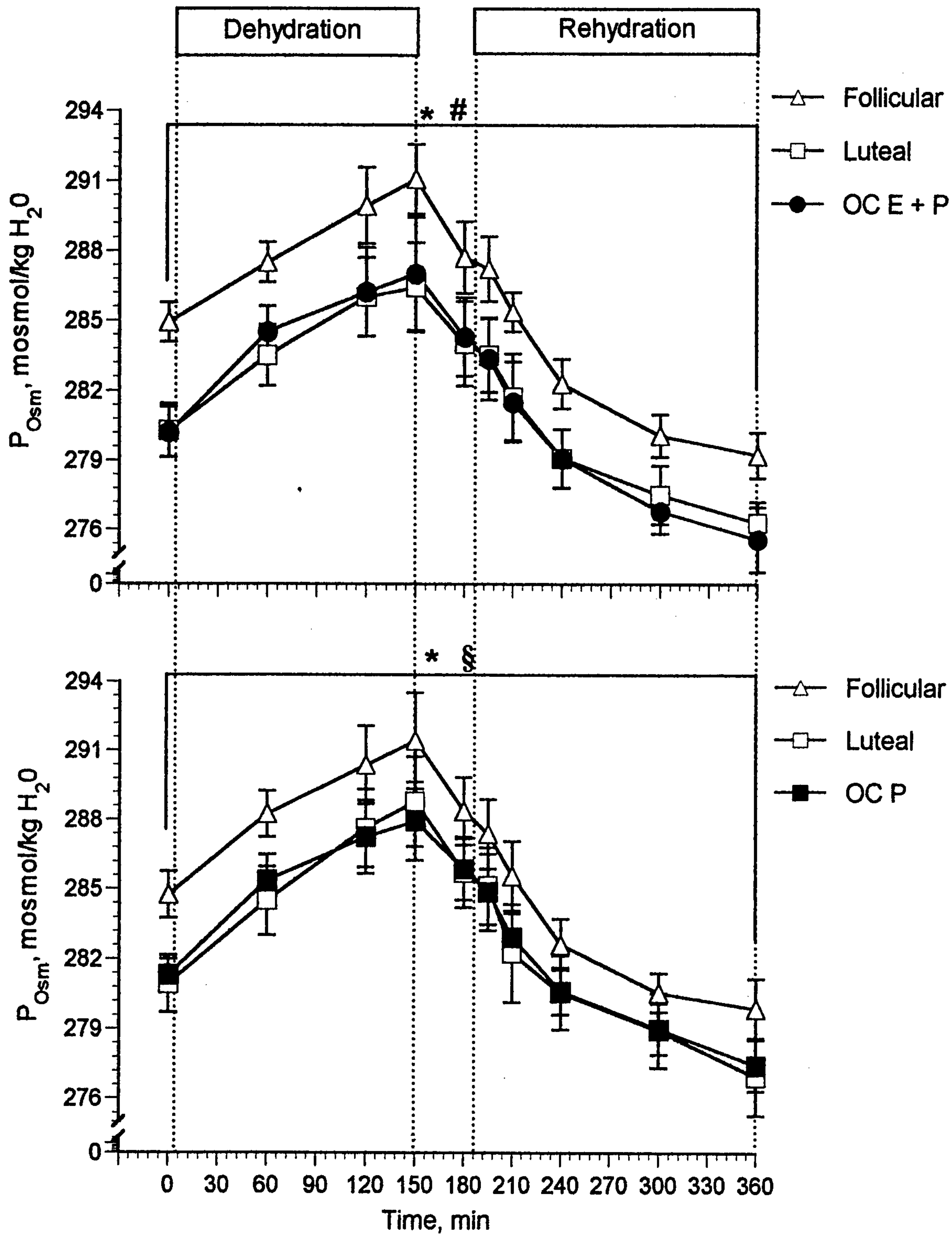

Figure 2. Plasma osmolality at rest, dehydration and rehydration

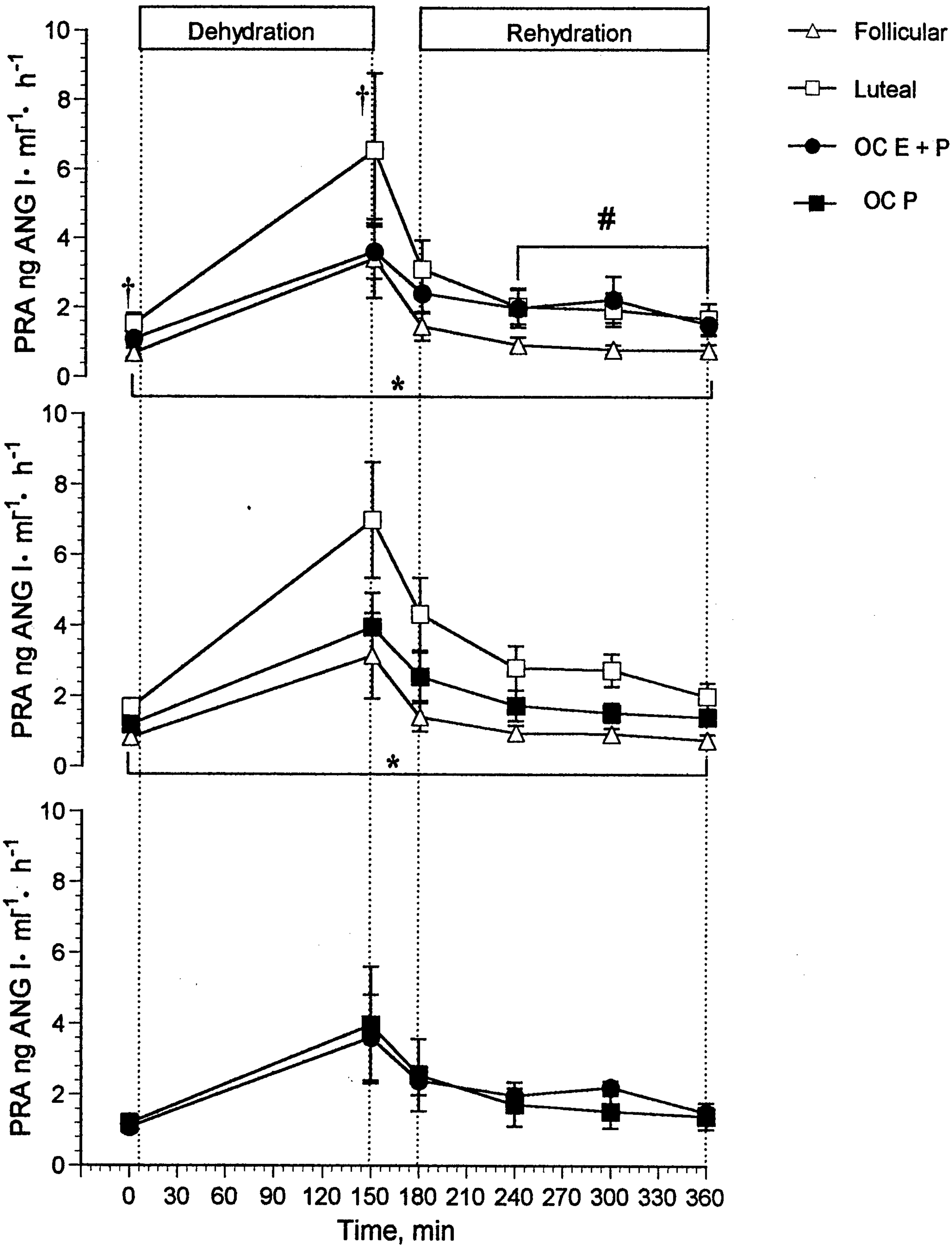

Figure 3. Plasma renin activity at rest, dehydration and rehydration

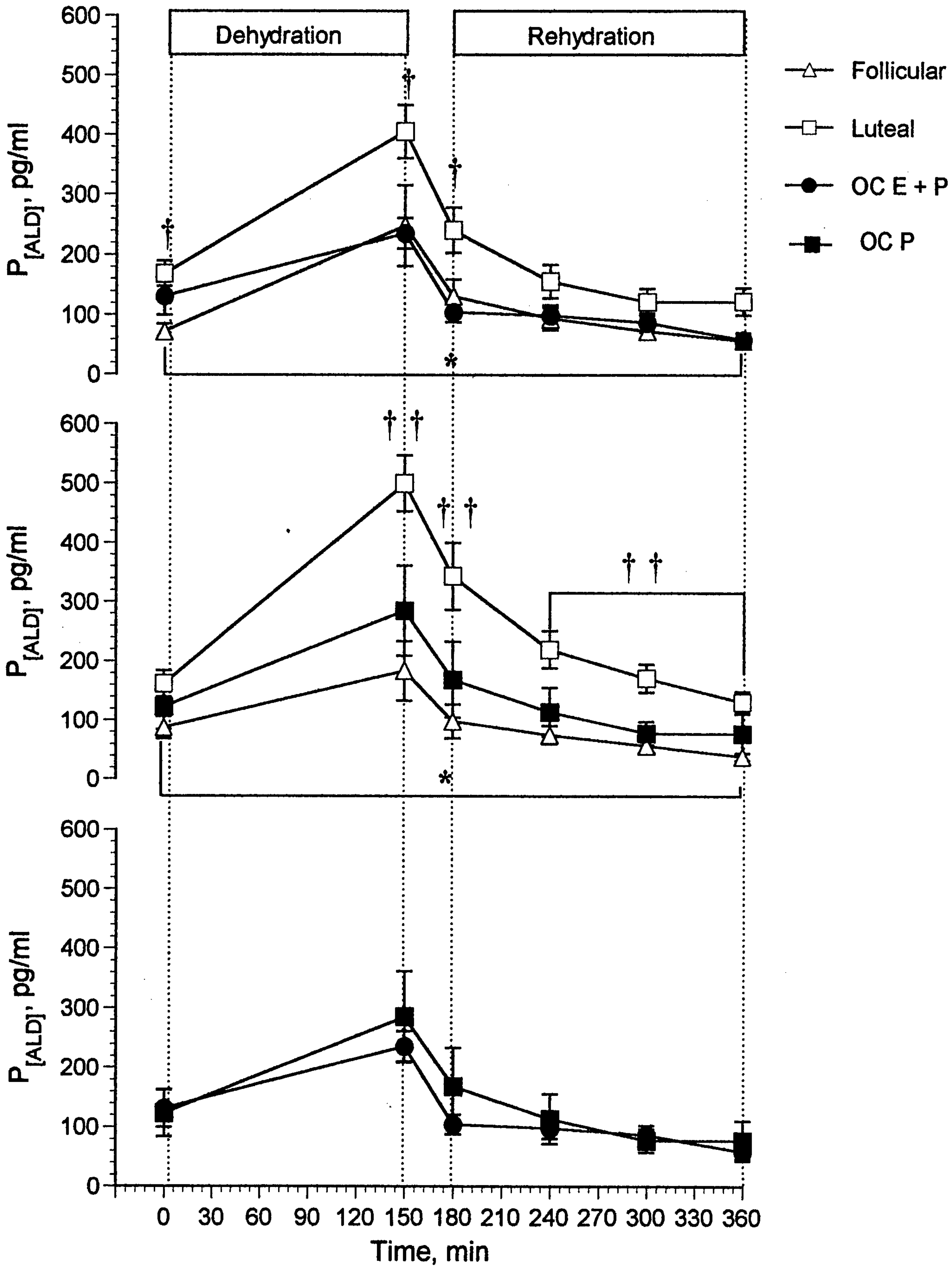

Figure 4. Plasma aldosterone concentration at rest, dehydration and rehydration

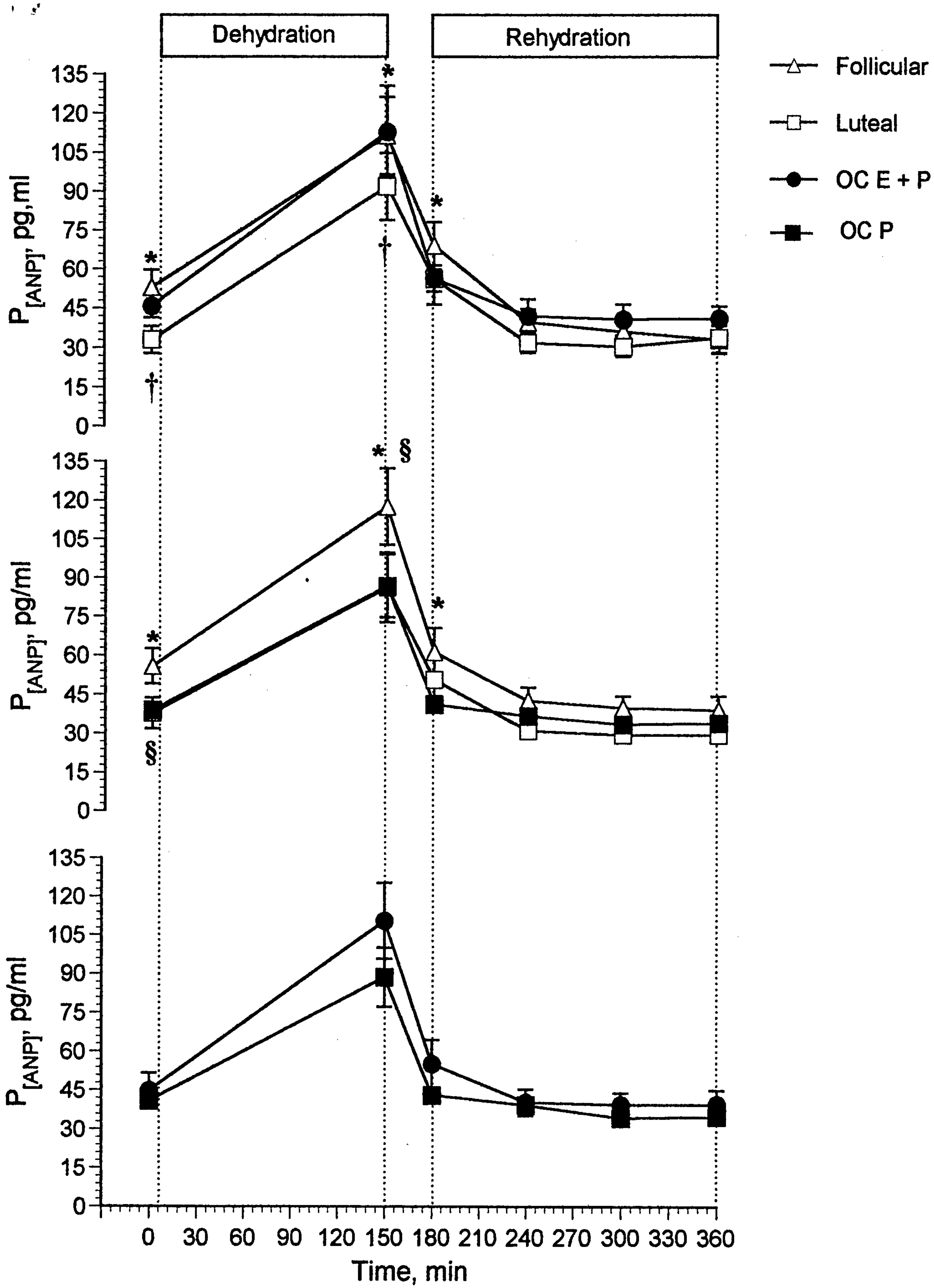

Figure 5. Plasma atrial natriuratic peptide concentration at rest, dehydration and rehydration

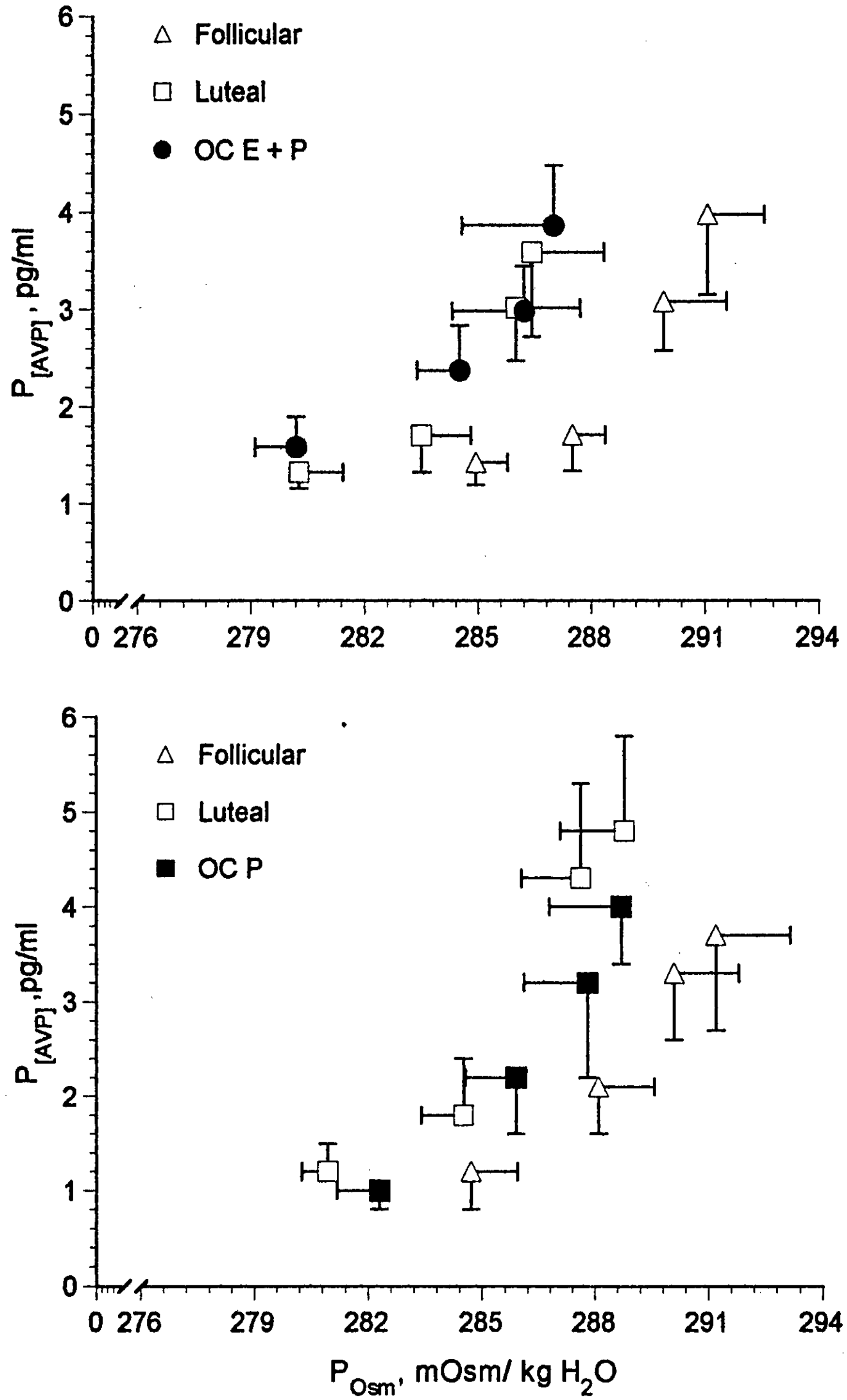

Figure 6. Osmotic regulation of arginine vasopressin during dehdyration.

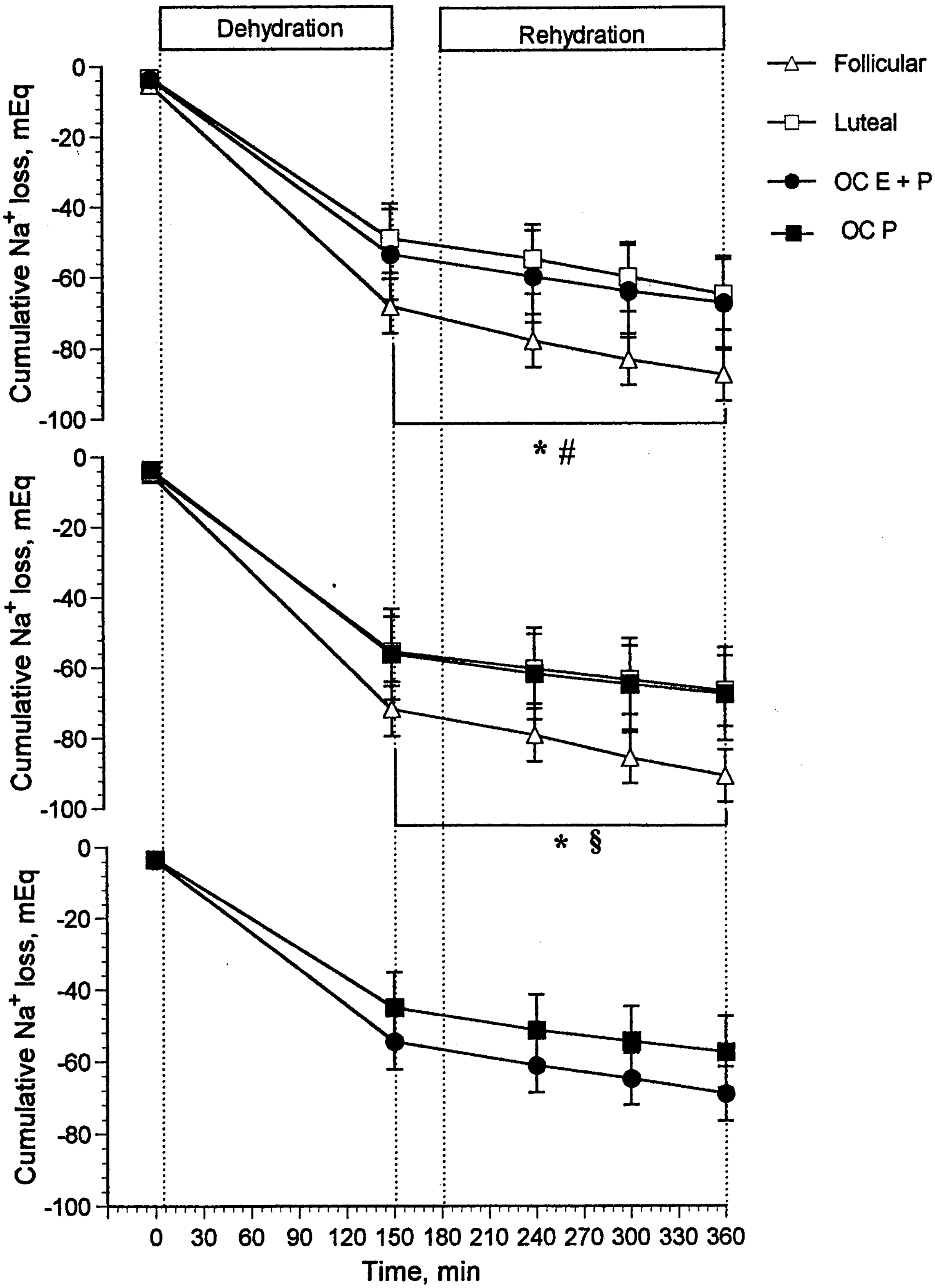

Figure 7. Cumulative sodium loss

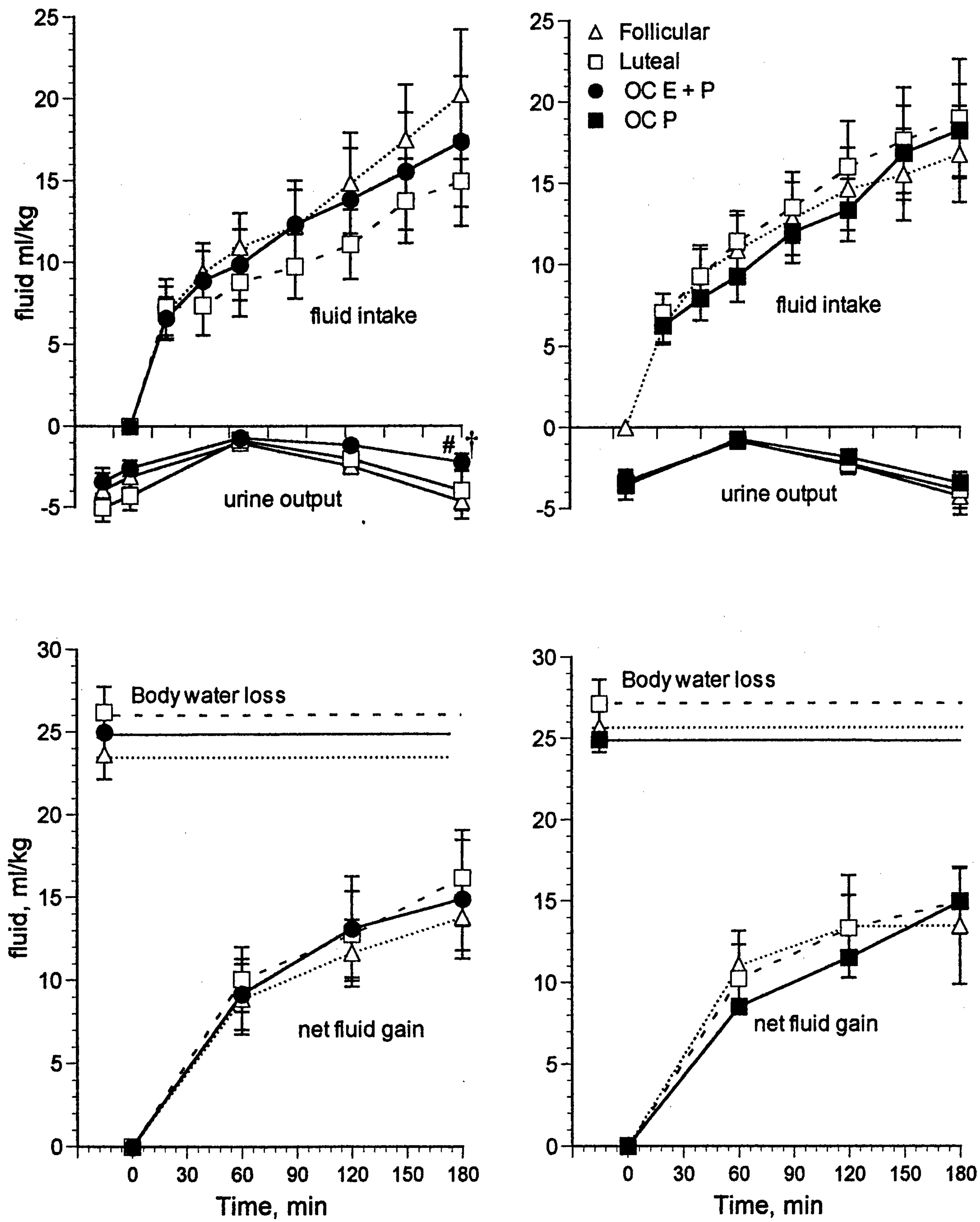

Figure 8. Body fluid balance

Physiological variability of fluid-regulation hormones in young women

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[1]*The John B. Pierce Laboratory, Departments of* [2]*Epidemiology and Public Health, and* [3]*Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06519; and* [4]*Women and Infants Hospital, Brown University School of Medicine, Providence, Rhode Island 02905*

Stachenfeld, Nina S., Loretta DiPietro, Cheryl A. Kokoszka, Celso Silva, David L. Keefe, and Ethan R. Nadel. Physiological variability of fluid-regulation hormones in young women. *J. Appl. Physiol.* 86(3): 1092–1096, 1999.—We tested the physiological reliability of plasma renin activity (PRA) and plasma concentrations of arginine vasopressin ($P_{[AVP]}$), aldosterone ($P_{[ALD]}$), and atrial natriuretic peptide ($P_{[ANP]}$) in the early follicular phase and midluteal phases over the course of two menstrual cycles (n = 9 women, ages 25 ± 1 yr). The reliability (Cronbach's $\alpha \geq 0.80$) of these hormones within a given phase of the cycle was tested *1*) at rest, *2*) after 2.5 h of dehydrating exercise, and *3*) during a rehydration period. The mean hormone concentrations were similar within both the early follicular and midluteal phase tests; and the mean concentrations of $P_{[ALD]}$ and PRA for the three test conditions were significantly greater during the midluteal compared with the early follicular phase. Although Cronbach's α for resting and recovery $P_{[ANP]}$ were high (0.80 and 0.87, respectively), the resting and rehydration values for $P_{[AVP]}$, $P_{[ALD]}$, and PRA were variable between trials for the follicular (α from 0.49 to 0.55) and the luteal phase (α from 0.25 to 0.66). Physiological reliability was better after dehydration for $P_{[AVP]}$ and PRA but remained low for $P_{[ALD]}$. Although resting and recovery $P_{[AVP]}$, $P_{[ALD]}$, and PRA were not consistent within a given menstrual phase, the differences in the concentrations of these hormones between the different menstrual phases far exceeded the variability within the phases, indicating that the low within-phase reliability does not prevent the detection of menstrual phase-related differences in these hormonal variables.

aldosterone; renin; atrial natriuretic peptide; arginine vasopressin; estrogen; progesterone

THE BODY'S WATER- AND SODIUM-regulating hormones vary considerably over the course of the menstrual cycle (7–9, 14, 15, 18, 19). For example, during the midluteal phase of the menstrual cycle, plasma aldosterone concentration ($P_{[ALD]}$) and plasma renin activity (PRA) are greater at rest (9) and during exercise (14, 15) than in the follicular phase. In addition, resting plasma arginine vasopressin concentration ($P_{[AVP]}$) is higher (8) during the preovulatory and midluteal phases of the cycle when plasma estrogen concentration ($P_{[E_2]}$) is high. In lower animals, estrogen administration increases osmotic stimulation of AVP (1, 4, 5) and water retention (3), and both estrogen and progesterone exhibit important effects on sodium regulation and the sodium-regulation hormones (10–12, 21); this supports the hypothesis that the gonadal steroids have important modulatory effects on body fluid and electrolyte balance.

No studies exist that examine the physiological reliability of the fluid-regulating hormones within a given phase and over the course of two or more menstrual cycles. Reported plasma concentrations of these hormones across different menstrual cycles differ due to natural physiological variations, due to selection of an inappropriate day to conduct physiological testing, due to variations in water and/or sodium intake, or due to inaccurate hormone-analysis techniques. The purpose of this study was to eliminate variability caused by the latter three reasons to determine the natural physiological variability of the responses of fluid- and sodium-regulating hormones over two menstrual cycles. Accordingly, we tested women twice during the early follicular phase (when estrogen and progesterone are low) and twice during the midluteal phase of the menstrual cycle (when estrogen and progesterone are high).

METHODS

Study Design

Subjects were nine healthy, nonsmoking women (age, 25 ± 1 yr; range, 22–31 yr). To drive the fluid-regulation system, each woman participated in a series of dehydration experiments in which the study hormones were measured *1*) at rest, *2*) during dehydration, and *3*) during rehydration in both the early follicular and the midluteal menstrual phases. The study design employed four dehydration experiments: two conducted in the early follicular phase (2–4 days after the beginning of menstrual bleeding) and two in the midluteal phase of the menstrual cycle (conducted 7–10 days after the luteinizing hormone peak), as determined individually by the use of ovulation-prediction kits (OvuQuick; Quidel, San Diego, CA). The tests were conducted during nonconsecutive men-

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strual phases, 12–16 wk apart. To verify the phase of the menstrual cycle, plasma levels of 17β-estradiol and progesterone were assessed from a basal blood sample.

Dehydration Experiments

On the day of the dehydration test, volunteers arrived at the laboratory between 7:00 and 8:00 AM, after they had eaten only a prescribed low-fat breakfast (~300 kcal). The subjects refrained from alcohol and caffeine for 12 h before the experiment. Subjects were asked to drink 7 ml/kg body weight of tap water at home before arrival at the laboratory. On arrival at the laboratory, the subjects gave a baseline urine sample; they were weighed and then sat on the contour chair of a cycle ergometer in the test chamber [27°C, 30% relative humidity (RH)] for 60 min of control rest. During the control period, an indwelling catheter was placed in an arm vein. At the end of the control period, a 20-ml blood sample was drawn and urine was collected. Consistency of the pretest hydration state was assessed from the specific gravity of the basal urine sample (mean = 1.001), which did not differ across trials.

After the control period, the chamber temperature was increased to 36°C. The subjects then exercised at 50% maximal power output for 150 min, with 5-min rest periods every 25 min, during which time they were deprived of fluids. Blood samples (10–20 ml) were drawn and body weight was assessed at 60, 120, and 150 min during exercise. At the end of exercise, the chamber temperature was reduced to 27°C. After dehydration, subjects rested for 30 min in a contour chair, without access to fluids; after 30 min, they drank water ad libitum for 180 min. Blood samples (20 ml) were taken just before drinking (*time 0*) and at 30, 60, 120, and 180 min of rehydration. Urine was collected at the end of exercise and at hourly intervals during rehydration, and the urine samples were analyzed for volume and sodium excretion.

Blood samples. Subjects were semirecumbent during placement of the catheter (21 gauge) and were seated for 60 min before samples were taken to ensure a steady state in plasma volume and constituents. Free-flowing venous blood was obtained for the measurement of hematocrit (Hct), plasma osmolality (P_{Osm}), PRA, $P_{[AVP]}$, $P_{[ALD]}$, $P_{[E_2]}$, and plasma concentrations of atrial natriuretic peptide ($P_{[ANP]}$) and progesterone ($P_{[P_4]}$). An aliquot (0.5 ml) was removed for immediate assessment of Hct in triplicate by microhematocrit. Second and third aliquots were transferred to a heparinized tube and a tube without additive, and all other aliquots were placed in tubes that contained EDTA. The tubes were centrifuged, and the plasma taken off the heparinized sample was analyzed for aldosterone. $P_{[E_2]}$ and $P_{[P_4]}$ were measured by using serum from the tube without additive. The EDTA samples were analyzed for $P_{[AVP]}$, $P_{[ANP]}$, and PRA. All blood samples were analyzed for Hct, P_{Osm}, $P_{[ALD]}$, $P_{[AVP]}$, $P_{[ANP]}$, and PRA; only the basal blood samples were also analyzed for $P_{[E_2]}$ and $P_{[P_4]}$.

Blood Analysis

P_{Osm} was measured by freezing-point depression (Advanced Instruments 3DII); $P_{[ALD]}$, $P_{[AVP]}$, $P_{[ANP]}$, $P_{[E_2]}$, and $P_{[P_4]}$ were measured by radioimmunoassay. Intra- and interassay coefficients of variation for the midrange standards were, respectively, as follows: $P_{[AVP]}$ (4.52 pg/ml), 6.0 and 3.4% [Immuno Biological Laboratories (IBL), Hamburg, Germany]; PRA (4.5 $ng \cdot ml^{-1}$ $ANG \cdot h^{-1}$), 2.3 and 2.9% (Diasorin, Stillwater, MN); $P_{[ALD]}$ (132 pg/ml), 3.4 and 3.6% (Diagnostic Products, Los Angeles, CA); $P_{[ANP]}$ (63.3 pg/ml), 5.1 and 5.2% (Diasorin); $P_{[E_2]}$ (64.3 pg/ml), 3.7 and 4.0% (Diagnostic Products); and $P_{[P_4]}$ (3.7 pg/ml), 2.1 and 2.5% (Diagnostic Products). The assay for AVP has a sensitivity of 0.8 pg/ml; this sensitivity is necessary to detect small, but important, changes in this hormone.

Statistical Analysis

Pearson's product-moment correlation on individual data was used to assess the slope and abscissal intercepts of the $P_{[AVP]}$-P_{Osm} relationship during dehydration (6). The within-phase reliability of our most important dependent variables (fluid-regulating hormones and osmotic regulation of AVP, as measured at rest, dehydration, and rehydration) was determined with Cronbach's α, assuming a value ≥0.80 as an acceptable level of reliability (2). Areas under the curve (AUC; trapezoid method) were calculated during the rehydration period (starting 30 min postexercise) for PRA, $P_{[ALD]}$, and $P_{[ANP]}$, and their reliability was determined within a given menstrual cycle by using Cronbach's α. We used repeated measures ANOVA models, followed by Bonferroni's *t*-test to test differences in the dependent variables both within and between menstrual phases. Data were analyzed by using BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and were expressed as means ± SE.

RESULTS

All subjects were tested during the first 5 days (4 ± 1 days) after the start of menstrual bleeding for early follicular-phase tests, and between 20 and 25 days (22 ± 2 days) for the midluteal-phase tests. Specifically, the subjects were tested between *days 7* and *10* after the LH peak, and, therefore, ~6–9 days after ovulation.

Between-Phase Measurements

At rest, Hct, $P_{[E_2]}$, $P_{[P_4]}$, $P_{[ALD]}$, and PRA were higher, and P_{Osm} and $P_{[ANP]}$ were lower, in the luteal phase compared with the follicular phase ($P < 0.05$); however, there were no differences in body weight or $P_{[AVP]}$ (Tables 1 and 2). During dehydrating exercise, body water loss (1.5 ± 0.2 kg, or 2.3% of preexercise body weight) was comparable between the follicular and midluteal phases. Similarly, despite the baseline variability, $P_{[AVP]}$ and PRA responses to exercise (i.e., change from baseline) were similar between the two phases (Table 2). However, this was not the case for $P_{[ALD]}$, in which the exercise response was greater during the midluteal phase. Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{Osm}, with *r* values ranging from 0.82 to 0.98. The abscissal intercept of the linear $P_{[AVP]}$-P_{Osm} relationship, or "theoretical osmotic threshold" for AVP release, was lower in the midluteal phase (278 ± 1 and 279 ± 1 mosmol/kgH_2O; Table 1 and Fig. 1) compared with the follicular phase (282 ± 1 and 283 ± 1 mosmol/kgH_2O; $P < 0.05$). The slopes of this relationship were unaffected by menstrual phase. During rehydration, the AUCs for $P_{[ALD]}$ and PRA were significantly greater in the luteal compared with the follicular phase.

Within-Phase Measurements

Early follicular phase. Within the follicular phase, there were no significant differences among the means

Table 1. *Subject characteristics in early follicular and midluteal phases of the menstrual cycle*

Characteristics	Follicular Phase	Luteal Phase
Body weight, kg		
Trial A	61.7 ± 3.6	61.3 ± 3.5
Trial B	61.5 ± 3.9	61.6 ± 3.7
Hematocrit, %		
Trial A	36.6 ± 0.8	36.8 ± 1.0*
Trial B	36.5 ± 0.7	37.9 ± 0.9*
$P_{[E_2]}$, pg/ml		
Trial A	26.9 ± 4.6	98.9 ± 16.6*
Trial B	20.7 ± 4.1	128.1 ± 20*
$P_{[P_4]}$, ng/ml		
Trial A	1.3 ± 0.4	8.7 ± 2.0*
Trial B	0.9 ± 0.4	9.8 ± 2.3*
P_{Osm}-$P_{[AVP]}$ slope, $pg \cdot ml^{-1} \cdot mosmol^{-1}$		
Trial A	0.47 ± 0.11	0.51 ± 0.18
Trial B	0.49 ± 0.14	0.56 ± 0.17
P_{Osm}-$P_{[AVP]}$ x-intercept, mosmol/kgH_2O		
Trial A	283 ± 2	279 ± 1*
Trial B	283 ± 1	279 ± 1*

Values are means ± SE; *Trial A* and *Trial B* are the first and second trials, respectively, within the specified menstrual phase. Preexercise body weight, hematocrit, and plasma concentrations of 17β-estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) in the early follicular and midluteal phases of the menstrual cycle. Slopes and abscissal intercepts are based on individual subjects' plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{Osm}) relationship during dehydration in the early follicular and midluteal phases of the menstrual cycle. *Significant difference between follicular and luteal phases, $P < 0.05$.

of any of the variables during rest, dehydration, and rehydration. However, with the exception of $P_{[ANP]}$, none of the resting values of the fluid-regulating hormones attained sufficiently high Cronbach's α to be considered reliable (Table 3). Reliability for $P_{[AVP]}$ and PRA was better after dehydrating exercise, although reliability remained low for $P_{[ALD]}$ (α = 0.66) and remained high for $P_{[ANP]}$ (α = 0.90). During dehydration, both the slope and abscissal intercept of the P_{Osm}-$P_{[AVP]}$ relationship were highly reliable within the follicular phase, attaining Cronbach's α of 0.96 and 0.90, respectively. Again, $P_{[AVP]}$, $P_{[ALD]}$, and PRA were not reliably reproduced during rehydration, whereas Cronbach's α for $P_{[ANP]}$ was 0.93. $P_{[E_2]}$ was highly reproducible within the follicular-phase tests, attaining Cronbach's α of 0.85, but $P_{[P_4]}$ attained a Cronbach's α value of only 0.62 between tests in the follicular phase.

Midluteal phase. As in the follicular phase, there were no differences in mean hormonal concentrations at rest, after dehydration, or during rehydration within the midluteal phase. Again, resting values for $P_{[AVP]}$, $P_{[ALD]}$, and PRA were not highly reproducible between the two midluteal phase tests (Table 3). Reliability for $P_{[ANP]}$ was greater, compared with the other fluid-regulating hormones, at rest and during exercise and rehydration. Despite high levels of reliability for osmotic regulation of AVP (Table 3), resting and rehydration levels of $P_{[AVP]}$ were not consistently correlated within the luteal-phase tests. In contrast to the follicular phase, however, both $P_{[E_2]}$ and $P_{[P_4]}$ were highly consistent between the two luteal-phase tests, yielding Cronbach's α values of 0.93 and 0.93, respectively.

DISCUSSION

We examined the within-phase physiological reliability of the fluid- and sodium-regulating hormone concentrations in the plasma over two nonconsecutive menstrual cycles (12–16 wk apart) during the early follicular and midluteal phases. $P_{[AVP]}$, $P_{[ALD]}$, and PRA varied within each of the different menstrual phases; however, there were no statistical differences among the means of any of these hormone concentrations. This indicates that the within-subject variability remains undetected when only the means are tested or reported. Nonetheless, our data indicate that between-phase differences in the hormone concentrations far exceed the variability within the phases, and, therefore, the low within-phase reliability does not prevent the detection of menstrual-phase-related changes in these variables. In contrast, $P_{[AVP]}$, PRA, and $P_{[ANP]}$ responses to dehydration were highly reliable within each menstrual phase; this indicates that hormonal responses to stress are

Table 2. *Fluid-regulation hormone concentrations at rest and during exercise and rehydration in early follicular and midluteal phases of menstrual cycle*

	Preexercise, 0 min	Exercise, 150 min	Rehydration, AUC
Follicular phase			
$P_{[ALD]}$, pg/ml			
Trial A	79 ± 12	275 ± 65	$228 \times 10^2 \pm 37 \times 10^2$
Trial B	96 ± 19	198 ± 47	$166 \times 10^2 \pm 30 \times 10^2$
PRA, $ng \cdot ml$ $ANG^{-1} \cdot h^{-1}$			
Trial A	0.8 ± 0.2	3.9 ± 1.0	287 ± 60
Trial B	0.9 ± 0.2	3.4 ± 1.1	267 ± 62
$P_{[AVP]}$, pg/ml			
Trial A	1.3 ± 0.2	3.7 ± 0.8	399 ± 72
Trial B	1.2 ± 0.4	3.5 ± 0.8	374 ± 106
$P_{[ANP]}$, pg/ml			
Trial A	33.0 ± 3.9	88.1 ± 11.7	$78 \times 10^2 \pm 8 \times 10^2$
Trial B	38.0 ± 5.3	87.9 ± 12.1	$76 \times 10^2 \pm 8 \times 10^2$
Luteal phase			
$P_{[ALD]}$, pg/ml			
Trial A	157 ± 22*	388 ± 43*	$330 \times 10^2 \pm 47 \times 10^2$ *
Trial B	155 ± 21*	500 ± 51*	$460 \times 10^2 \pm 52 \times 10^2$ *
PRA, $ng \cdot ml$ $ANG^{-1} \cdot h^{-1}$			
Trial A	1.8 ± 0.4*	6.1 ± 1.7*	471 ± 113*
Trial B	1.7 ± 0.2*	4.2 ± 0.9*	653 ± 121*
$P_{[AVP]}$, pg/ml			
Trial A	1.2 ± 0.2	3.2 ± 0.6	347 ± 79
Trial B	1.1 ± 0.3	3.7 ± 1.1	496 ± 125
$P_{[ANP]}$, pg/ml			
Trial A	49.6 ± 5.6	109.2 ± 14.5	$94 \times 10^2 \pm 9 \times 10^2$
Trial B	54.6 ± 9.2	114.8 ± 22.2	$101 \times 10^2 \pm 14 \times 10^2$

Values are means ± SE. *Trials A* and *B* are first and second trials, respectively, within specified menstrual phase. AUC, area under the curve (trapezoid). Plasma renin activity (PRA), $P_{[AVP]}$, and plasma concentrations of aldosterone ($P_{[ALD]}$) and atrial natriuretic peptide ($P_{[ANP]}$) at rest, and in response to dehydrating exercise and 180 min of ad libitum rehydration in early follicular and midluteal phases of menstrual cycle. *Significant difference between follicular and luteal phases, $P < 0.05$.

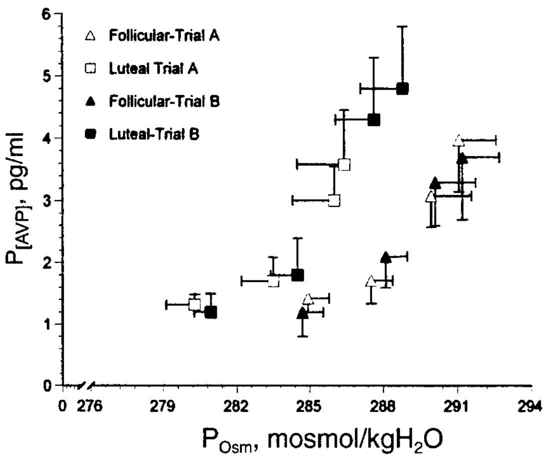

Fig. 1. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in plasma osmolality (P_{Osm}) during dehydration in follicular and luteal phase tests. *Trials A* and *B* are the first and second trials, respectively, within the specified menstrual phases. Data are means ± SE.

more consistent, despite the variability in baseline values.

Although there were no significant within-phase differences between the means of the sodium-regulating hormones, only $P_{[ANP]}$ values were consistently reliable during rest, exercise, and rehydration within either of the two phases. Resting $P_{[AVP]}$, $P_{[ALD]}$, and PRA were quite variable across the two trials within both the follicular and luteal menstrual phases. Indeed, this baseline variability exists even with careful control of predehydration water and sodium intake, posture, and timing of the experiments to coincide with specific events during the menstrual cycle (such as ovulation and menses). Resting or basal variations in $P_{[AVP]}$ may be exaggerated further by the fact that values were close to the lowest level of sensitivity of our assay technique (i.e., 0.8 pg/ml). Also, because the rehydration was ad libitum, hydration-recovery rates may have been different among the test days. Therefore, although

Table 3. *Cronbach's α for reliability within 2 follicular and 2 luteal phase tests*

	Cronbach's α	
	Follicular phase	Luteal phase
Resting $P_{[AVP]}$	0.49	0.25
Exercise $P_{[AVP]}$	0.81*	0.98*
Rehydration $P_{[AVP]}$	0.58	0.96*
$P_{[AVP]}$-P_{Osm} slope	0.96*	0.81*
$P_{[AVP]}$-P_{Osm} intercept	0.90*	0.86*
Resting $P_{[ANP]}$	0.80*	0.80*
Exercise $P_{[ANP]}$	0.90*	0.87*
Rehydration $P_{[ANP]}$	0.93*	0.80*
Resting PRA	0.49	0.51
Exercise PRA	0.72	0.89*
Rehydration PRA	0.67	0.95*
Resting $P_{[ALD]}$	0.55	0.66
Exercise $P_{[ALD]}$	0.66	0.82*
Rehydration $P_{[ALD]}$	0.64	0.76
Resting $P_{[E_2]}$	0.85*	0.93*
Resting $P_{[P_4]}$	0.62	0.92*

*Cronbach's α ≥ 0.80 was considered reliable.

total fluid intake was similar over the four tests, changes in drinking patterns or drinking rates may substantially affect AVP release at a given blood sampling point (17) and, consequently, affect our ability to observe repeatable $P_{[AVP]}$.

In any case, despite the low within-phase reliability of $P_{[AVP]}$ at rest and during rehydration, osmotic regulation of $P_{[AVP]}$ (i.e., slopes and intercepts) during dehydration was highly reproducible. This indicates that, although individual values may vary, the regulation of this hormone in response to environmental stress (e.g., exercise) remains constant. This is an important finding, because small shifts in the regulation of AVP lead to large changes in renal water retention (13). Moreover, although a number of studies have demonstrated changes in osmotic regulation of $P_{[AVP]}$ over the course of a single menstrual cycle (18, 19), the menstrual-phase effects on the P_{Osm} threshold for AVP release are only ~5–6 mosmol/kgH_2O, making essential a precise and consistent measurement of the P_{Osm} intercept within a given menstrual phase.

Interestingly, the shifts in osmotic regulation of AVP and the fluid regulation hormones over the course of the menstrual cycle do not seem to impact overall body fluid and sodium retention. Despite the shift in osmotic AVP regulation, fluid loss during exercise was similar in both menstrual phases. In the luteal phase, a progesterone-induced inhibition of aldosterone-dependent sodium reabsorption at distal sites in the nephron causes transient natriuresis (12). This natriuresis is followed by a compensatory stimulation of the renin-aldosterone system (9, 16, 20), resulting in a slight attenuation of sodium excretion during the luteal phase (7.2 ± 1.4 vs. 11.5 ± 2.0 meq). Nonetheless, overall water and sodium balance appear unaffected by the shifts in either progesterone or the sodium regulation hormones (9). This leads us to speculate that estrogen and progesterone have their primary impact on body water regulation through changes in body water and sodium distribution rather than through retention.

We also tested the reliability of the female sex hormones 17β-estradiol and progesterone. $P_{[E_2]}$ was highly reproducible between the two trials in both the follicular and midluteal phases. $P_{[P_4]}$, although reproducible during the luteal phase, was somewhat variable between the two trials in the follicular phase. $P_{[P_4]}$ is normally low during the follicular phase of the menstrual cycle, so even small variations lead to large error values and may thus exaggerate the variability of $P_{[P_4]}$ during the follicular phase. Nonetheless, despite the low reliability, $P_{[P_4]}$ values were consistent and low enough to indicate the subjects were in the follicular phase of the menstrual cycle.

The variability in the fluid-regulating hormones was not substantial enough either to create significant statistical differences in means between trials within the same menstrual phase or to obscure the large differences in these hormone concentrations between menstrual phases. Nonetheless, these findings suggest that there is a natural variability in these hormone responses, which may be undetected when only grouped mean values are presented.

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

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Effects of oral contraceptives on body fluid regulation

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Stachenfeld, Nina S., Celso Silva, David L. Keefe, Cheryl A. Kokoszka, and Ethan R. Nadel. Effects of oral contraceptives on body fluid regulation. *J. Appl. Physiol.* 87(3): 1016–1025, 1999.—To test the hypothesis that estrogen reduces the operating point for osmoregulation of arginine vasopressin (AVP), thirst, and body water balance, we studied nine women (25 ± 1 yr) during 150 min of dehydrating exercise followed by 180 min of ad libitum rehydration. Subjects were tested six different times, during the early-follicular (twice) and midluteal (twice) menstrual phases and after 4 wk of combined [estradiol-norethindrone (progestin), OC E + P] and 4 wk of norethindrone (progestin only, OC P) oral contraceptive administration, in a randomized crossover design. Basal plasma osmolality (P_{osm}) was lower in the luteal phase (281 ± 1 mosmol/kgH_2O, combined means, $P < 0.05$), OC E + P (281 ± 1 mosmol/kgH_2O, $P < 0.05$), and OC P (282 ± 1 mosmol/kgH_2O, $P < 0.05$) than in the follicular phase (286 ± 1 mosmol/kgH_2O, combined means). High plasma estradiol concentration lowered the P_{osm} threshold for AVP release during the luteal phase and during OC E + P [*x*-intercepts, 282 ± 2, 278 ± 2, 276 ± 2, and 280 ± 2 mosmol/kgH_2O, for follicular, luteal (combined means), OC E + P, and OC P, respectively; $P < 0.05$, luteal phase and OC E + P vs. follicular phase] during exercise dehydration, and 17β-estradiol administration lowered the P_{osm} threshold for thirst stimulation [*x*-intercepts, 280 ± 2, 279 ± 2, 276 ± 2, and 280 ± 2 mosmol/kgH_2O for follicular, luteal, OC E + P, and OC P, respectively; $P < 0.05$, OC E + P vs. follicular phase], without affecting body fluid balance. When plasma 17β-estradiol concentration was high, P_{osm} was low throughout rest, exercise, and rehydration, but plasma arginine vasopressin concentration, thirst, and body fluid retention were unchanged, indicating a lowering of the osmotic operating point for body fluid regulation.

estrogen; progesterone; rehydration; osmolality; arginine vasopressin

ESTROGEN ADMINISTRATION can lead to significant body fluid retention (19) and, in very high doses, hypertension (14). Although the mechanism underlying the estrogen-mediated body fluid retention is unclear, a number of studies have demonstrated that the osmotic thirst and arginine vasopressin (AVP) responses to hypertonicity occur earlier with elevations in estrogen and progesterone, such as during the luteal phase of the menstrual cycle (18, 26, 27) and during pregnancy (8). Using hypertonic saline infusion followed by water loading, Vokes et al. (27) demonstrated a downward resetting of the osmoreceptors during the luteal phase. In addition, we recently demonstrated a reduction in the osmotic threshold for AVP release during hypertonic saline infusion in postmenopausal women who were taking estrogen (19), and the greater AVP response was associated with fluid retention.

Although it seems clear that elevations in estrogen, with and without elevations in progesterone, alter osmotic regulation of AVP (18, 26, 27) and thirst sensitivity (8, 27), the specific estrogen effects on body fluid regulation after body water loss are not known. Addressing the question of estrogen effects during dehydration as opposed to hypertonic saline infusion is important, because hypertonic saline infusion increases plasma osmolality (P_{osm}) and volume (PV), whereas dehydration increases P_{osm} while it reduces total body water and PV. The AVP-P_{osm} and thirst-P_{osm} relationships are shifted with differing volume status (15), so an evaluation of the fluid regulation systems while PV is reduced and the body is actively retaining fluid is necessary to fully understand the effects of estrogen on these systems. These differences in PV status during hypertonic saline infusion and dehydration may exaggerate AVP and thirst responses to osmotic stimulation during dehydration but may also have particular relevance during subsequent rehydration, inasmuch as they could alter the compartmentalization of ingested fluid. Alterations in the compartmentalization of ingested fluid have important implications for physical performance, because changes in body water storage will influence fluid maintenance during exertion and fluid restoration during recovery from exertion in environmentally stressful conditions.

Therefore, to determine estrogen effects on the body water regulation system, we administered oral contraceptives to young women and then evaluated their responses to progressive, exercise-induced dehydration and a subsequent rehydration period. Combined oral contraceptive agents deliver pharmacological levels of estrogens that exhibit 6–10 times the estrogenic activity provided by endogenous, circulating estrogens. In contrast, progestin-only pills contain no estrogen, and the unopposed progestin tends to downregulate estrogen receptors. Thus these two oral contraceptive preparations differ significantly in their estrogenic activity, providing the appropriate conditions in which to isolate estrogen effects on body fluid regulation. We hypoth-

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esized that oral contraceptive pills containing estrogen would reduce the threshold for osmotic AVP and thirst increases to progressive, exercise-induced dehydration to a greater degree than a progestin-only pill. In addition, we hypothesized that fluid intake and renal water retention would also be increased and lead to greater water retention during combined oral contraceptive treatment. Finally, consideration of the PV and arterial pressure control of Na^+ excretion is essential for a complete evaluation of body fluid regulation, so we also determined the effects of our oral contraceptive regimen on Na^+ regulation and the Na^+-regulating hormones.

METHODS

Study design. Subjects were nine healthy, nonsmoking women (age 25 ± 1 yr, range 22–31 yr) with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, had medical and gynecological examinations, and provided written confirmation of a negative Papanicolaou smear within 1 yr of being admitted to the study. During the month (early-follicular phase) preceding the first dehydration experiment, resting PV was determined with Evans blue dye dilution (see below) and peak O_2 consumption was determined from an incremental cycle ergometer test with use of an automated metabolic cart (Sensor Medics, Yorba Linda, CA).

Each woman participated in two series of experiments (Fig. 1), each consisting of two baseline dehydration tests (4 total) and one dehydration test while taking each type of oral contraceptive (2 total). Estrogen and progesterone vary across the menstrual cycle, so the study design employed two dehydration baseline studies conducted in the early-follicular phase, 2–4 days after the beginning of menstrual bleeding (low estrogen and progesterone), one for each pill treatment, and two conducted in the midluteal phase, 7–9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel, San Diego, CA). After completing the first baseline dehydration tests, the subjects again performed dehydration protocols after 4 wk of continuous combined (estrogen-progestin, OC E + P) or progestin-only oral contraceptive treatment (random assignment, double blind, OC P). After completing the first dehydration testing series and after a 4-wk "washout" period, the subjects crossed over to the other pill treatment.

During OC E + P treatment, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. During OC P treatment, subjects received 1 mg/day of the progestin norethindrone. To verify phase of the menstrual cycle and compliance to the pill regimen, plasma levels of estrogen and progesterone were assessed from the preexercise blood sample before the dehydration protocol was undertaken.

Dehydration experiments. Volunteers arrived at the laboratory between 7 and 8 AM, after having eaten only a prescribed low-fat breakfast (~300 kcal). The subjects refrained from alcohol and caffeine for 12 h before the experiment. Blood volumes were not manipulated before any of the experiments, although subjects prehydrated by drinking 7 ml/kg body wt of tap water at home before arrival at the laboratory. On arriving at the laboratory, each subject gave a baseline urine sample, was weighed to the nearest 10 g on a beam balance, and then sat on the contour chair of a semirecumbent cycle ergometer in the test chamber (27°C, 30% relative humidity) for 60 min of control rest. During the control period, an indwelling catheter (21 gauge) was inserted into an arm vein, and electrodes and a blood pressure cuff were placed. Subjects were semirecumbent during placement of the catheter and were seated for 60 min before sampling to ensure a steady state in PV and constituents. Resting blood pressure (Colin Medical Instruments, Komaki, Japan) and heart rate (electrocardiogram) were recorded at the end of the 60-min control period. At the end of the control period, a blood sample (20 ml) was drawn and urine was collected. Hydration state was assessed from the specific gravity of the preexercise urine sample (mean = 1.002 ± 0.001).

After the control period the chamber temperature was increased to 36°C and the subjects began pedaling at an intensity corresponding to 50% maximal power output. The exercise duration was 150 min, with 5-min rest periods every 25 min, during which time they received no fluids. Blood samples (10–20 ml) were drawn and body weight was measured immediately before the rest periods at 60, 120, and 150 min during exercise. On the basis of previous experience in our laboratory, we expected a weight loss of 2.0–2.5% of preexercise body weight. To accurately determine weight loss, we previously determined the saturated weight of the shorts and a jog-bra worn during exercise (0.250 kg) and subtracted this weight from the final exercise weight. Heart rate was monitored throughout exercise to ensure subject safety. A urine sample was collected at the end of exercise, and then the chamber temperature was reduced to 27°C for the 3.5-h recovery period.

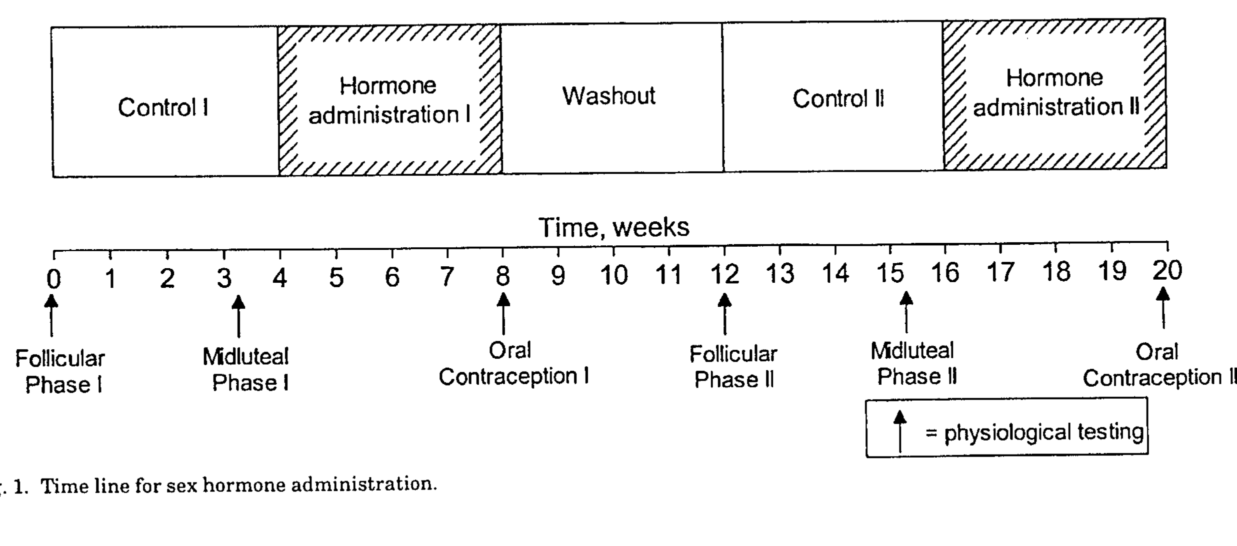

Fig. 1. Time line for sex hormone administration.

For sweat collection during exercise, sealed absorbent patches (Pacific Biometrics, Seattle, WA) were placed on the thigh, forearm, chest, back, and forehead for 20- to 25-min periods. The sweat patch consisted of 4.7 × 3.1-cm filter paper, sealed and affixed to the skin with Tegaderm. The skin areas used for the patch were cleaned with deionized water before placement of the patch and wiped with a clean dry towel. Local sweat rate was determined by each patch weight increase (to 0.0001 g) from the dry weight per minute on the skin. After sweat was collected and the sweat patch was weighed, the sweat-soaked patches were transferred to plastic screw-capped bottles. The fluid in the patches was collected by centrifugation with use of nylon Microfuge centrifuge filter tubes and analyzed for Na^+ and K^+ concentrations.

After dehydration, each subject rested for 30 min in a contour chair without access to fluids to allow the body fluid compartments to stabilize, then drank water ad libitum for 180 min. Blood was sampled just before drinking (*time 0*, 10 ml) and at 15 (10 ml), 30, 60, 120, and 180 min of rehydration (20 ml each sample). Urine samples were collected and body weight was measured every 60 min of rehydration. The total blood drawn during each experiment was ~180 ml, which is too small to have any independent effect on any of the measured variables.

All blood samples were analyzed for hematocrit (Hct), concentrations of Hb ([Hb]) and total protein ([TP]), P_{osm}, plasma concentrations of creatinine, glucose, urea, and AVP ($P_{[AVP]}$), and serum concentrations of Na^+ ($S_{[Na^+]}$) and K^+ ($S_{[K^+]}$). Plasma renin activity (PRA) and concentrations of aldosterone ($P_{[ald]}$) and atrial natriuretic peptide ($P_{[ANP]}$) were analyzed from the control sample, from samples taken at the end of exercise, and from samples taken at 0, 60, 120, and 180 min of rehydration. The control blood samples were also analyzed for 17β-estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$).

Blood and urine analysis. An aliquot (1 ml) was removed for immediate assessment of Hct, [Hb], and [TP] in triplicate by microhematocrit, cyanomethemoglobin, and refractometry, respectively. A second aliquot was transferred to a heparinized tube, and a third aliquot was placed into a tube without anticoagulant for the determination of $S_{[Na^+]}$ and $S_{[K^+]}$. All other aliquots were placed in chilled tubes containing EDTA. The tubes were immediately centrifuged at 4°C, and the plasma taken off the heparinized sample was analyzed for creatinine and aldosterone. The samples containing EDTA were analyzed for $P_{[AVP]}$, $P_{[ANP]}$, and PRA. These centrifuged samples were frozen immediately and stored at −80°C until analysis. All urine samples were analyzed for volume, osmolality, and creatinine concentration.

Plasma, sweat, and urine Na^+ and K^+ were measured by flame photometry (model 943, Instrumentation Laboratory). P_{osm} and urine osmolality were assessed by freezing-point depression (model 3DII, Advanced Instruments). Plasma and urine creatinine, plasma glucose, and urea concentrations were determined by colorimetric assay (Sigma Diagnostic Products). $P_{[AVP]}$, $P_{[ald]}$, $P_{[ANP]}$, $P_{[E_2]}$, $P_{[P_4]}$, and PRA were measured by RIA. Intra- and interassay coefficients of variation for the midrange standard were as follows: 6.0 and 3.4% (Immuno Biological Laboratories, Hamburg, Germany) for $P_{[AVP]}$ (4.52 pg/ml), 3.4 and 3.6% (Diagnostic Products, Los Angeles, CA) for $P_{[ald]}$ (132 pg/ml), 5.1 and 5.2% (Diasorin, Stillwater, MN) for $P_{[ANP]}$ (63.3 pg/ml), 3.7 and 4.0% (Diagnostic Products) for $P_{[E_2]}$ (64.3 pg/ml), 2.1 and 2.5% (Diagnostic Products) for $P_{[P_4]}$ (3.7 pg/ml), and 2.3 and 2.9% (Diasorin) for PRA (4.5 $ng \cdot ml\ ANG^{-1} \cdot h^{-1}$). The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

Blood volume. Absolute blood volume was measured by dilution of a known amount of Evans blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing (10, 20, and 30 min). PV was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, with 1.5% lost from the circulation within the first 10 min taken into account. Blood volume was calculated from PV and Hct concentration corrected for peripheral sampling (9).

Thirst ratings. We assessed thirst perception by asking the subject to make a mark on a line rating scale in response to the question, "How thirsty do you feel now?" The line is 175 mm long and is marked "not at all" on one end and "extremely thirsty" at the 125-mm point. We told subjects that they could mark beyond the "extremely thirsty" point if they wished and they could even have extended the line if they felt it was necessary. This method was developed by Marks et al. (11) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and P_{osm} during hypertonic saline infusion and dehydration in young subjects (20, 25).

Calculations. Total water loss due to dehydration was determined from body weight loss during exercise. Net fluid gain during rehydration was calculated by subtracting total urine loss from water intake, with the assumption that respiratory and sweat losses were negligible in the 27°C recovery condition. Changes in PV were estimated from changes in Hct and [Hb] from the control (preexercise) sample according to the equation

$$\%\Delta PV = 100\{[(Hb_b)/(Hb_a)] \cdot [(1 - Hct_a \cdot 10^{-2})]/[(1 - Hct_b \cdot 10^{-2})]\} - 100$$

in which subscripts *a* and *b* denote measurements at *time a* and control, respectively.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na^+}) were calculated from the following equations

$$FE_{H_2O} = (U_V/GFR) \cdot 100$$

$$FE_{Na^+} = (U_V \cdot U_{[Na^+]}/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \cdot S_{[Na^+]}$$

in which the subscript f is glomerular filtrate, U_v is urine flow rate, $U_{[Na^+]}$ is Na^+ concentration in urine, and $S_{[Na^+]}$ is $S_{[Na^+]}$ in protein-free solution (meq/kgH_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Electrolyte losses in sweat and urine during dehydration were calculated by multiplying the volume of water loss in each fluid by the concentration of the electrolyte within the fluid. Whole body sweat electrolyte concentration was calculated from sweat rate, local electrolyte concentration, and body surface area using the following equation (24)

$$[E]_m = (0.07[E]_{fh}SR_{fh} + 0.36[E]_{tr}SR_{tr} + 0.13[E]_{fa}SR_{fa} + 0.32[E]_{th}SR_{th}/0.07SR_{fh} + 0.36SR_{tr} + 0.13SR_{fa} + 0.32SR_{th})$$

where the subscripts m, fh, tr, fa, and th are whole body mean, forehead, trunk, forearm, and thigh, respectively, [E] is electrolyte concentration (Na^+ or K^+, meq/l), SR is local sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$), and the constants 0.07, 0.36, 0.13, and 0.32 represent the percent distribution of body surface in the head, trunk, arms, and legs, respectively. Total electrolyte loss from sweat was calculated by multiplying $[E]_m$ by total body sweat loss, calculated from the change in body weight during exercise. Electrolyte losses during rehydration were calculated by multiplying the volume of water loss by the concentration of electrolytes in the urine.

Statistics. Separate repeated-measures ANOVA models were performed to test differences in the dependent variables due to menstrual phase and OC E + P or OC P administration. Bonferroni's *t*-test was used to correct for multiple comparisons where appropriate. Pearson's product moment correlation was used to assess the relationship of $P_{[AVP]}$ as a function of P_{osm} on individual data during exercise, and the abscissal intercepts defined the "theoretical osmotic threshold" for AVP release (8). We used repeated-measures ANOVA models, followed by Bonferroni's *t*-test, to test differences in the abscissal intercepts and slopes due to menstrual phase or oral contraceptive treatment (4, 8). On the basis of an α-level of 0.05 and a sample size of 8, our β-level (power) was ≥0.80 for detecting effect sizes of 2.0 pg/ml, 0.67 ml/min, 2.0 ng·ml $ANG^{-1} \cdot h^{-1}$, 40 pg/ml, 10 pg/ml, and 3.0 meq for $P_{[AVP]}$, renal free water clearance, PRA, $P_{[ald]}$, $P_{[ANP]}$, and renal Na^+ excretion, respectively (4, 7, 8, 28). Data were analyzed using BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and expressed as means ± SE.

RESULTS

Combined oral contraceptive administration caused severe nausea in one woman, and she did not complete dehydration testing while on this pill, so all her control data for OC E + P have also been excluded. This analysis compares the dehydration test responses of nine women on OC P with their two control tests and eight women on OC E + P with their control tests.

Subject characteristics. The subjects were 25 ± 1 yr (range 20–34 yr), weighed 62.5 ± 3.6 kg, and were 164 ± 3 cm tall. Their mean blood volume was 66. 4 ± 2.0 ml/kg, mean PV was 2,780 ± 124 ml, and mean peak O_2 consumption was 30.6 ± 2.4 $ml \cdot kg^{-1} \cdot min^{-1}$.

Baseline (preexercise). Preexercise body weight was similar for both phases of the menstrual cycle and oral contraceptive administration (Table 1). The $P_{[E_2]}$ and $P_{[P_4]}$ values in Table 1 demonstrate that the subjects were tested in the early-follicular and midluteal phases of the menstrual cycle during both trials. Finally, oral contraceptive administration suppressed the endogenous production of 17β-estradiol and progesterone (Table 1).

Preexercise P_{osm} was lower in the luteal phase and after 1 mo of OC E + P and OC P than in the follicular phase (Fig. 2; $P < 0.05$), although $P_{[AVP]}$ and thirst were unaffected by phase of the menstrual cycle or by oral contraceptive administration (Table 2). Plasma glucose and urea concentrations were unaffected by menstrual phase or either oral contraceptive pill, but $S_{[Na^+]}$ was lower [138 ± 0.5, 136 ± 0.4, 136.2 ± 0.6, and 136.6 ± 0.3 meq/l for follicular and luteal phases (combined means), OC E + P, and OC P, respectively], suggesting that the lower P_{osm} (in the luteal phase and with oral contraceptives) was a function of lower $S_{[Na^+]}$. Changes in Hct and [Hb] indicated an estimated (calculated) contraction of PV compared with the follicular phase (Table 1). There was no effect of menstrual phase or oral contraceptive treatment on plasma protein concentration (6.7, 6.8, 6.7, and 6.8 g/l for follicular and luteal phases, OC E + P, and OC P, respectively). Preexercise PRA was greater in both luteal phase tests than in the follicular phase tests and during OC E + P, and $P_{[ald]}$ was increased in the luteal phase tests compared with the follicular phase tests (Table 3; $P < 0.05$). In contrast, $P_{[ANP]}$ was greater at baseline in the follicular phase tests than in the luteal phase and during OC P, and $P_{[ANP]}$ was greater during OC E + P than in the luteal phase test (Table 3; $P < 0.05$). Preexercise U_v, urine osmolality, GFR, and renal electrolyte excretion were similar within subjects before each exercise test.

Preexercise heart rate and blood pressure were similar at baseline and dehydration within the follicular and luteal phase tests, so the combined mean of the two series is given for the baseline values and for the dehydration tests. Baseline heart rate and mean blood pressure were unaffected by menstrual phase, averaging 78 ± 4 beats/min and 85 ± 2 mmHg during the

Table 1. *Subject characteristics and changes in osmotic AVP and thirst regulation*

	Follicular Phase ($n = 8$)	Midluteal Phase ($n = 8$)	OC E + P ($n = 8$)	Follicular Phase ($n = 9$)	Midluteal Phase ($n = 9$)	OC P ($n = 9$)
Body wt, kg	61.4 ± 4.1	61.8 ± 4.1	61.6 ± 3.8	60.7 ± 3.7	61.1 ± 3.4	60.0 ± 3.5
PV, ml	2780 ± 124					
$P_{[E_2]}$, pg/ml	27.3 ± 5.6	105.1 ± 26.2	<12.0	26.1 ± 6.7	146.7 ± 38.3	25.1 ± 5.3
	(12.3–40.8)	(63.6–189.6)		(13.1–36.2)	(61.1–222.0)	(6.4–26.7)
$P_{[P_4]}$, ng/ml	1.3 ± 0.6	8.7 ± 3.1	<0.02	0.49 ± 1.0	9.8 ± 2.2	<0.02
	(0.3–2.2)	(5.2–19.1)		(0.4–0.8)	(5.2–18.3)	
P_{osm}-$P_{[AVP]}$ slope, $pg \cdot ml^{-1} \cdot mosmol^{-1}$	0.47 ± 0.11	0.51 ± 0.18	0.49 ± 0.12	0.49 ± 0.14	0.55 ± 0.17	0.46 ± 0.14
P_{osm}-$P_{[AVP]}$ *x*-intercept, mosmol/kg H_2O	282 ± 1	278 ± 1*	276 ± 2†	283 ± 1	279 ± 1*	280 ± 2
P_{osm}-thirst slope, mm/mosmol	13.7 ± 3.5	14.0 ± 2.7	13.3 ± 3.7	12.8 ± 1.7	12.9 ± 2.9	13.7 ± 2.1
P_{osm}-thirst *x*-intercept, mm	280 ± 3	278 ± 2	276 ± 2†	280 ± 1	279 ± 2	280 ± 2
PV change, %		−8.4 ± 2.5	3.2 ± 2.1		−7.5 ± 2.7	−2.3 ± 2.5

Values are means ± SE; ranges are in parentheses. Resting plasma volume (PV) was measured on a separate day in the follicular phase. Preexercise body weight and plasma concentrations of endogenous 17β-estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) were measured in early-follicular and midluteal phases and during administration of combined [estradiol + progestin (norethindrone), OC E + P] and progestin (norethindrone)-only (OC P) oral contraceptive pills. Slopes and abscissal intercepts of individual subject's plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{osm}) and thirst-P_{osm} relationships during dehydration in early-follicular and midluteal phases and OC E + P and OC P are shown. Percent change in PV relative to the follicular phase was estimated from changes in preexercise hematocrit and Hb. * $P < 0.05$, follicular vs. midluteal phase; † $P < 0.05$, follicular phase vs. OC E + P.

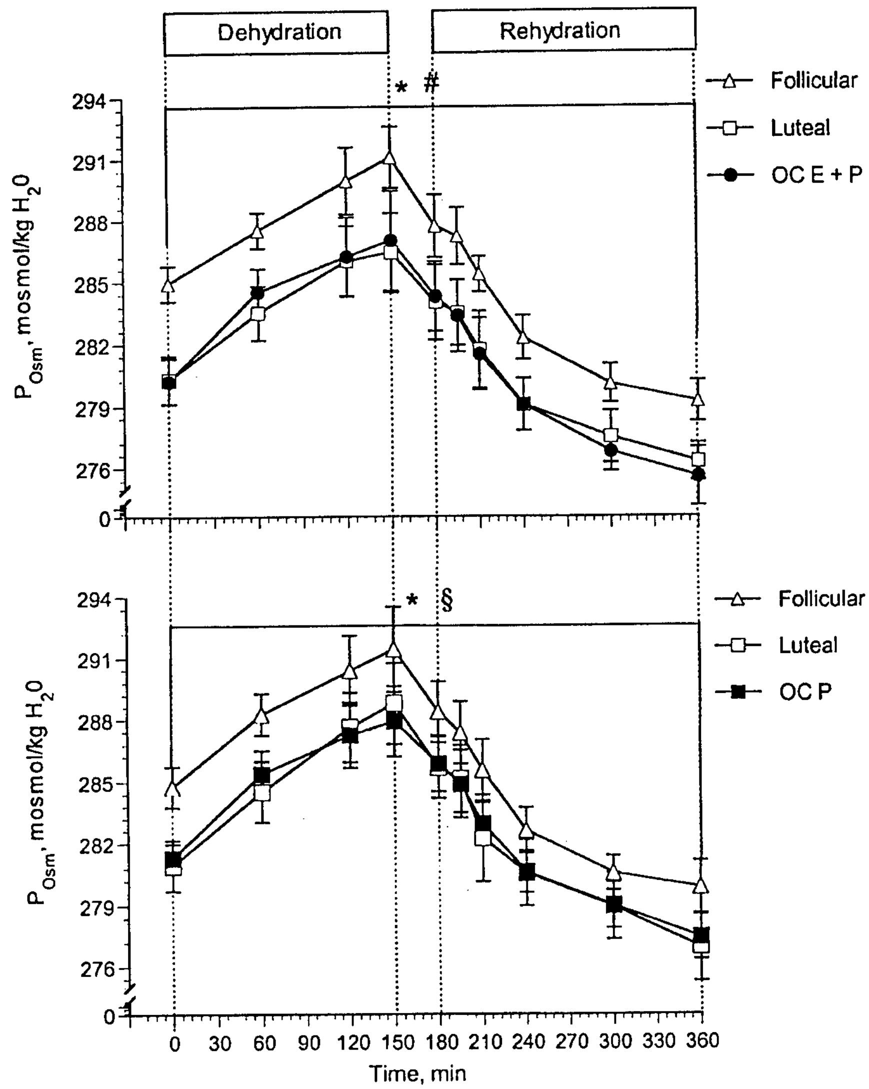

Fig. 2. Plasma osmolality (P_{osm}) at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in follicular and luteal phases and during combined estradiol-progestin (norethindrone, OC E + P, n = 8) and progestin (norethindrone)-only oral contraception administration (OC P, n = 9). $^*P <$ 0.05, follicular vs. luteal phase. $^{\#}P <$ 0.05, follicular phase vs. OC E + P. $^{\S}P <$ 0.05, follicular phase vs. OC P. Values are means ± SE.

follicular phase and 78 ± 5 beats/min and 82 ± 2 mmHg during the luteal phase. These cardiovascular variables were also unchanged by oral contraceptive treatment, averaging 78 ± 3 beats/min and 83 ± 1 mmHg and 81 ± 2 beats/min and 81 ± 2 mmHg during OC E + P and OC P, respectively.

Exercise responses. The subjects lost similar body weight (and percent body weight) at the end of 150 min of exercise during the follicular (1.4 ± 0.1 kg, 2.3%) and luteal (1.4 ± 0.1 kg, 2.2%) phase tests and during OC E + P (1.3 ± 0.2 kg, 2.3%). The same was true for the follicular (1.4 ± 0.1 kg, 2.3%) and luteal (1.4 ± 0.1 kg, 2.4%) phase tests compared with OC P (1.3 ± 0.1 kg, 2.2%). Heart rate increased to similar levels during dehydrating exercise in the follicular (145 ± 6 beats/min) and luteal (141 ± 5 beats/min) phase tests and during the OC P test (141 ± 7 beats/min), but this increase was attenuated during the OC E + P test (135 ± 6 beats/min, $P < 0.05$). Mean blood pressure did not change during dehydration in any of the experimental conditions.

Exercise increased P_{osm} and $P_{[AVP]}$ and decreased PV similarly during the follicular and luteal phases and during OC E + P and OC P (Fig. 2, Table 2). Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{osm} (mean r = 0.88 ± 0.03). The abscissal intercepts of the linear $P_{[AVP]}$-P_{osm} relationship, or "theoretical osmotic threshold" for AVP release, was significantly lower in the midluteal phase and with OC E + P than in the follicular phase (Table 1, $P <$ 0.05). The slopes of this relationship, however, were unaffected by menstrual phase or oral contraceptive use. Figure 3 shows the downward shift in the linear $P_{[AVP]}$-P_{osm} relationships during dehydrating exercise when $P_{[E_2]}$ and $P_{[P_4]}$ were increased in the luteal phase and during OC E + P. The data in Table 2 indicate that thirst increased similarly during dehydration in all conditions. Linear regression analysis of the individual subjects' P_{osm} and thirst responses indicated significant correlations (mean r = 0.90 ± 0.03). Osmotic thirst

Table 2. *Plasma AVP concentrations, subjective thirst responses, and PV changes*

	Preexercise (0 min)	End Exercise (150 min)	Rehydration 0 min	60 min	120 min	180 min
			OC E+P			
$P_{[AVP]}$, pg/ml						
Follicular	1.3 ± 0.2	4.0 ± 0.8	3.3 ± 0.9	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3
Luteal	1.2 ± 0.2	3.8 ± 0.7	3.0 ± 0.7	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.4
OC E+P	1.6 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	2.7 ± 0.7	1.9 ± 0.4	2.3 ± 0.4
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	111 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC E+P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6
PV, % change						
Follicular		−8.6 ± 1.3	−2.6 ± 1.6	1.3 ± 1.6	1.2 ± 1.7	2.5 ± 1.8
Luteal		−9.5 ± 2.6	−3.3 ± 2.0	0.2 ± 1.4	0.7 ± 1.6	0.5 ± 1.5
OC E+P		−7.9 ± 1.2	−0.5 ± 1.2	1.9 ± 1.3	3.6 ± 1.0	5.1 ± 1.7
			OC P			
$P_{[AVP]}$, pg/ml						
Follicular	1.2 ± 0.4	3.7 ± 1.0	2.5 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	1.6 ± 0.4
Luteal	1.1 ± 0.3	4.8 ± 1.4	2.3 ± 0.6	2.0 ± 0.5	1.9 ± 0.6	1.9 ± 0.6
OC P	1.0 ± 0.2	4.0 ± 1.2	2.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.5 ± 0.4
Thirst, mm						
Follicular	20 ± 5	97 ± 12	90 ± 12	17 ± 6	12 ± 4	9 ± 4
Luteal	28 ± 9	97 ± 6	98 ± 11	31 ± 10	21 ± 6	25 ± 10
OC P	24 ± 8	97 ± 9	108 ± 6	19 ± 9	19 ± 9	18 ± 9
PV, % change						
Follicular		−7.5 ± 1.2	0.0 ± 1.4	2.3 ± 1.1	3.1 ± 1.1	5.0 ± 0.7
Luteal		−7.4 ± 1.0	0.1 ± 1.1	3.2 ± 0.1	0.8 ± 1.2	1.6 ± 1.1
OC P		−6.5 ± 1.0	0.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.3	5.2 ± 1.6

Values are means ± SE. Anginine vasopressin plasma concentration ($P_{[AVP]}$), cognitive thirst ratings, and plasma volume (PV) (estimated percent change from preexercise value) were measured at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in follicular and luteal phases and during OC E + P (n = 8) and OC P (n = 9).

stimulation was unaffected by menstrual phase, but OC E + P led to a fall in the abscissal intercept of this relationship (Table 1).

PRA, $P_{[ald]}$, and $P_{[ANP]}$ increased during exercise in all conditions, with luteal phase values for $P_{[ald]}$ remaining above the follicular phase, OC E + P, and OC P (Table 3; $P < 0.05$). Sweat Na^+ loss was greatest during exercise in the follicular phase tests (56.3 ± 7.0 and 59.4 ± 9.2 meq, $P < 0.05$) but was similar between the luteal phase tests (45.2 ± 9.1 and 46.5 ± 7.8 meq) compared

Table 3. *Plasma concentrations of Na^+-regulating hormones*

	Preexercise (0 min)	End Exercise (150 min)	Rehydration 0 min	60 min	120 min	180 min
			OC E+P			
PRA, $ng \cdot ml\ ANG^{-1} \cdot h^{-1}$						
Follicular	0.7 ± 0.1[a]	3.4 ± 1.2[a]	1.5 ± 0.4[a]	0.9 ± 0.2[a,b]	0.8 ± 0.1[a,b]	0.8 ± 0.2[a,b]
Luteal	1.5 ± 0.3[c]	6.5 ± 2.2[c]	3.1 ± 0.8	2.0 ± 0.5	1.9 ± 0.5	1.7 ± 0.5
OC E+P	1.1 ± 0.2	3.6 ± 0.8	2.4 ± 0.6	2.0 ± 0.6	2.2 ± 0.7	1.5 ± 0.3
$P_{[ald]}$, pg/ml						
Follicular	72 ± 12[a]	247 ± 67[a]	131 ± 27[a]	94 ± 19[a]	73 ± 14[a]	56 ± 11[a]
Luteal	168 ± 20	405 ± 45[c]	240 ± 38[c]	155 ± 27	122 ± 22	123 ± 22[c]
OC E+P	131 ± 32	235 ± 26	104 ± 17	98 ± 17	87 ± 16	58 ± 11
$P_{[ANP]}$, pg/ml						
Follicular	52.9 ± 6.8[a]	111.5 ± 14.8[a]	69.1 ± 9.2[a]	39.7 ± 5.0	36.3 ± 4.4	33.3 ± 5.2
Luteal	33.0 ± 5.1[c]	91.8 ± 13.0[c]	56.1 ± 9.8	31.9 ± 3.7	30.6 ± 3.8	33.7 ± 5.7
OC E+P	45.8 ± 4.5	113.0 ± 17.5	56.4 ± 5.4	42.2 ± 6.4	40.8 ± 5.8	41.1 ± 4.7
			OC P			
PRA, $ng \cdot ml\ ANG^{-1} \cdot h^{-1}$						
Follicular	0.8 ± 0.2[a]	3.2 ± 1.2[a]	1.4 ± 0.4[a]	1.0 ± 0.2[a]	0.9 ± 0.2[a]	0.8 ± 0.2[a]
Luteal	1.7 ± 0.3	7.0 ± 1.6	4.3 ± 1.0	2.8 ± 0.2	2.7 ± 0.5	2.0 ± 0.4
OC P	1.2 ± 0.2	4.0 ± 1.0	2.6 ± 0.7	1.7 ± 0.4	1.5 ± 0.3	1.4 ± 0.2
$P_{[ald]}$, pg/ml						
Follicular	88 ± 19[a]	184 ± 41[a]	98 ± 28[a]	75 ± 15[a]	57 ± 12[a]	38 ± 7[a]
Luteal	162 ± 22	500 ± 47[d]	343 ± 57[d]	220 ± 31[d]	171 ± 24[d]	131 ± 18[d]
OC P	123 ± 40	285 ± 41	169 ± 44	114 ± 32	78 ± 20	77 ± 22
$P_{[ANP]}$, pg/ml						
Follicular	55.8 ± 10.3[a,e]	117.6 ± 25.0[a,e]	61.4 ± 8.8[a,e]	42.7 ± 5.7	40.0 ± 4.3	39.2 ± 4.1
Luteal	37.8 ± 6.0	86.3 ± 13.6	50.7 ± 8.0	31.2 ± 3.0	29.6 ± 2.3	29.6 ± 3.0
OC P	38.9 ± 2.8	86.9 ± 12.1	41.3 ± 3.2	36.7 ± 2.0	33.8 ± 2.3	34.2 ± 2.5

Values are means ± SE. Plasma renin activity (PRA) and plasma aldosterone ($P_{[ald]}$) and atrial natriuretic peptide concentrations ($P_{[ANP]}$) were measured at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in the follicular and luteal phases and during OC E + P (n = 8) and OC P (n = 9). [a] $P < 0.05$, follicular vs. luteal phase; [b] $P < 0.05$, follicular phase vs. OC E + P; [c] $P < 0.05$, luteal phase vs. OC E + P; [d] $P < 0.05$, luteal phase vs. OC P; [e] $P < 0.05$, follicular phase vs. OC P.

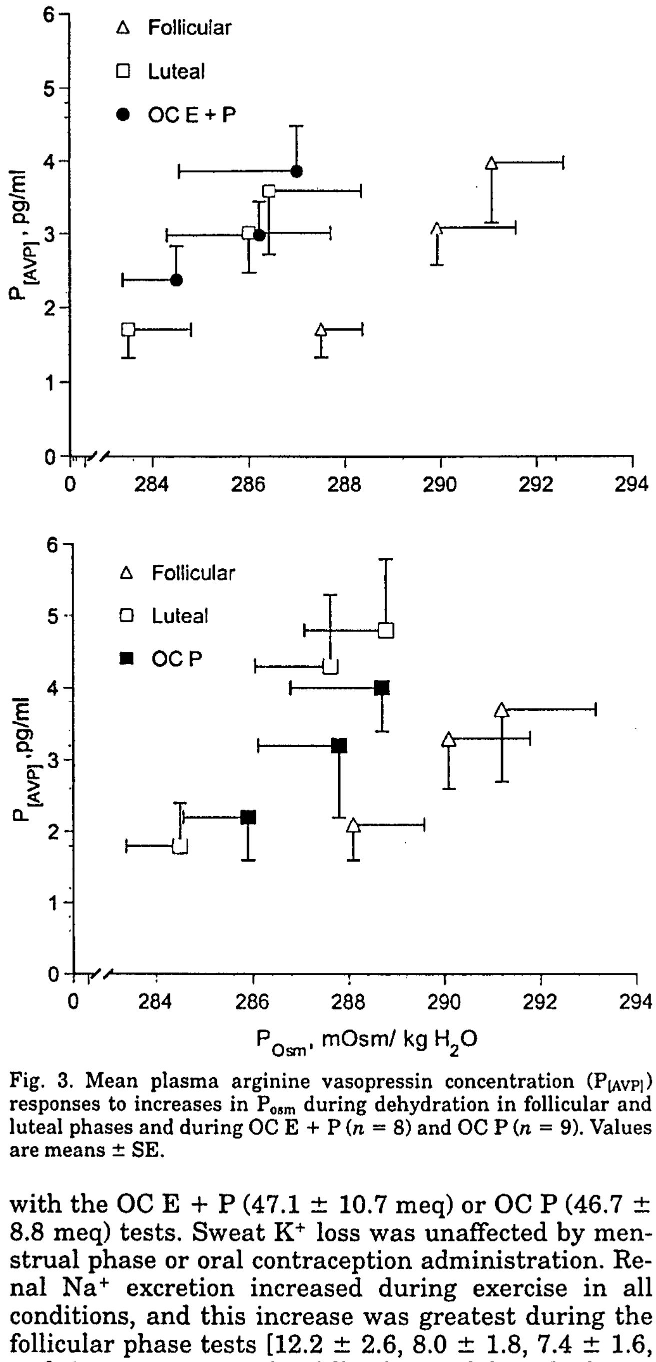

Fig. 3. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in P_{osm} during dehydration in follicular and luteal phases and during OC E + P (n = 8) and OC P (n = 9). Values are means ± SE.

with the OC E + P (47.1 ± 10.7 meq) or OC P (46.7 ± 8.8 meq) tests. Sweat K^+ loss was unaffected by menstrual phase or oral contraception administration. Renal Na^+ excretion increased during exercise in all conditions, and this increase was greatest during the follicular phase tests [12.2 ± 2.6, 8.0 ± 1.8, 7.4 ± 1.6, and 8.5 ± 3.3 meq for follicular and luteal phases (combined means), OC E + P, and OC P, respectively, $P < 0.05$].

Rehydration. Ad libitum fluid intake was similar by the end of the 180 min of rehydration on all six experimental test days. At 180 min of ad libitum drinking, subjects had restored 41 ± 5 and 40 ± 10% (follicular phase), 42 ± 7 and 39 ± 6% (luteal phase), 38 ± 11% (OC E + P), and 39 ± 7% (OC P) of body weight that was lost during dehydration. P_{osm} was higher throughout the rehydration period in the follicular phase than in the luteal phase, OC E + P, and OC P tests (Fig. 2; $P < 0.05$), although $P_{[AVP]}$ was similar during all rehydration tests. For the entire rehydration period, PRA was lower during the follicular phase tests than during the luteal phase tests, and $P_{[ald]}$ was significantly greater in the luteal phase tests than in the follicular phase and the OC P test (Table 3, $P < 0.05$).

During rehydration, neither renal function nor electrolyte excretion was affected by menstrual phase or oral contraceptive administration, and overall fluid balance (i.e., fluid intake − urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration (Fig. 4). Heart rate recovered to similar levels during rehydration in the follicular (75 ± 4 beats/min) and luteal (79 ± 4 beats/min) phase tests and during the OC E + P (78 ± 4 beats/min) and OC P (83 ± 4 beats/min) tests. Mean blood pressure remained unchanged throughout rehydration (78 ± 2, 79 ± 4, 77 ± 2, and 79 ± 2 mmHg for follicular and luteal phases, OC E + P, and OC P, respectively).

DISCUSSION

Our major finding was that administration of oral contraceptive pills containing estrogen increased osmotically induced AVP and thirst stimulation during dehydration in young, healthy women, although there were no changes in body fluid regulation during dehydration or subsequent ad libitum rehydration. These findings indicate that the shift in osmotic regulation of AVP and thirst represents a shift in body water regulation to a lower P_{osm} operating point. These data extend to young women our earlier findings in postmenopausal women, in whom estrogen administration reduced the P_{osm} threshold for AVP release during hypertonicity (19), although with an important difference. In postmenopausal women, 17β-estradiol administration reduced the P_{osm} threshold for AVP release during hypertonicity but also increased water retention and, therefore, did not indicate a shift in the operating point for body fluid regulation. In contrast, estradiol administration to the young women in our present investigation reduced P_{osm} but did not affect $P_{[AVP]}$, thirst ratings, or body water loss. After dehydration the subjects restored body water to the reduced, preexercise levels of P_{osm} during ad libitum rehydration, indicating a shift in the operating point for body fluid volume and composition with increased blood levels of estrogen. During dehydrating exercise, Na^+ excretion was lower during the luteal phase and OC E + P and OC P than during the follicular phase. However, although $P_{[ald]}$ and PRA were greater at rest and during rehydration in the luteal phase, neither the estrogen nor the progestin (norethindrone) in oral contraceptives stimulated the renin-angiotensin-aldosterone system or increased Na^+ retention or blood pressure.

Vokes et al. (27) used hypertonic saline infusion and water loading to stimulate and suppress the osmoreceptors, respectively, and demonstrated a resetting of osmoreceptor thresholds for AVP and thirst in the luteal phase of the menstrual cycle. Our findings support those of Vokes et al. and others (18, 26), indicating that AVP secretion persists at lower P_{osm}

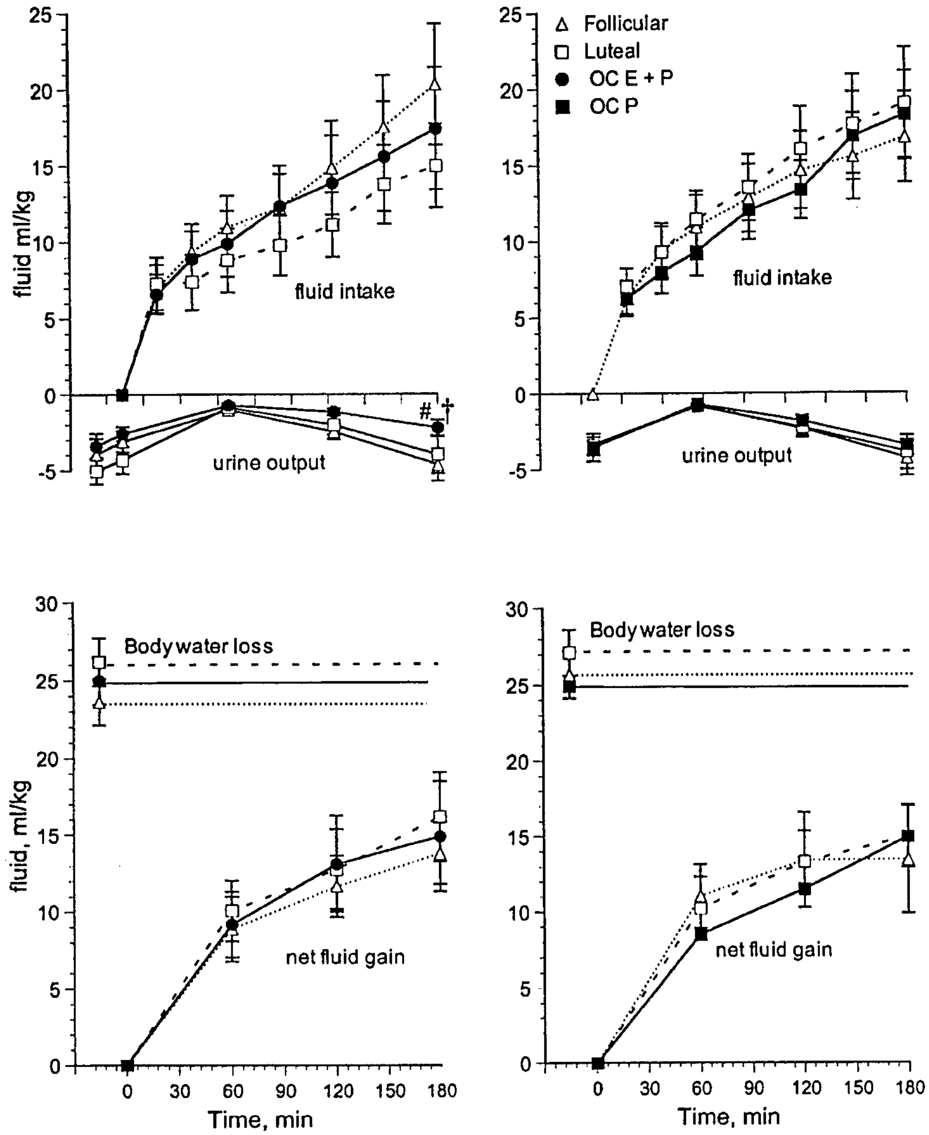

Fig. 4. Body fluid balance after dehydrating exercise and during 180 min of ad libitum rehydration in the follicular and luteal phases and during OC E + P (n = 8) and OC P (n = 9). #$P < 0.05$, follicular phase vs. OC E + P. †$P < 0.05$, luteal phase vs. OC E + P. Values are means ± SE.

during the luteal phase, thus causing a reduction in renal free water excretion and maintenance of this lower plasma tonicity. Estrogen and progesterone are elevated in the midluteal phase, so these studies did not determine whether the changes in osmoregulation were due to estrogen or progesterone effects. The data in our investigation extend these earlier findings and suggest that the shift in osmoregulation is due to the estrogen component of the oral contraceptive pill, because this shift did not occur during administration of progestin (norethindrone) only, which not only contains no estradiol, but downregulates estrogen receptors (17). Furthermore, progestin does not have a strong impact on estrogenic activity when administered with estradiol because of weak binding of progestins to estrogen receptors (17).

Estrogen readily crosses the blood-brain barrier and can likely modulate osmotic AVP and thirst regulation via its action within the central nervous system. Studies in lower animals have demonstrated that estrogen acts directly on estrogen-binding neurons in the hypothalamus (1, 2, 5, 16), thereby affecting synthesis and release of AVP. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (16), and osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the supraoptic nucleus of brain slices of ovariectomized rats (2). Estrogen may also modulate hypothalamic AVP release indirectly through catecholaminergic (10) and/or angiotensinergic (23) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [^{3}H]estradiol, Heritage et al. (10) identified estradiol-binding sites in the nuclei of catecholamine neuronal systems, as well as the presence of catecholamine nerve terminals surrounding estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (6) noted parallel changes in brain norepinephrine and AVP in normally cycling rats and that ovarian steroids modulated norepinephrine turnover in the paraventricular nucleus, indicating that estrogen may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (23).

Conversely, peripheral mechanisms for the estrogen effect on osmotic stimulation of AVP are unlikely to play a role in the response. For example, PV reduction, such as that during the midluteal phase, could have contributed to the lower P_{osm} threshold for AVP release, because PV is a potent AVP stimulus. However, this mechanism seems unlikely, because there was no change in PV during OC E + P relative to the follicular phase. In addition, the luteal phase PV contraction was not associated with a great enough fall in PV (<10%) to stimulate AVP (15). ANP has also been shown to suppress the osmotically induced rise in AVP (3), but the follicular phase and OC E + P were associated with greater $P_{[ANP]}$, although with different osmotic AVP response.

Blood volume and arterial pressure also play important roles in body fluid regulation, primarily by modulating Na^+ excretion. Previously, oral contraceptives containing high doses of estrogen (2 mg/day) led to hypertension and greater plasma angiotensinogen levels, although with only small elevations in plasma renin or aldosterone levels (14). The estrogen dose in our study did not increase blood pressure or cause consistent elevations in PRA and aldosterone, and norethindrone (the progestin in OC P), a progestational derivative of testosterone without antimineralocorticoid properties, also had no effect on PV. Nonetheless, our data confirm earlier findings demonstrating PV contraction during the midluteal phase of the menstrual cycle during rest, exercise, and heat exposure (21, 22), as well as large elevations in the sodium-regulating hormones (12). During the luteal phase a progesterone-mediated inhibition of aldosterone-dependent Na^+ reabsorption at distal sites in the nephron produces a transient natriuresis (13) and a compensatory stimulation of the renin-aldosterone system (12). The renin and aldosterone stimulation is a component of a system evolved to maintain blood pressure and plasma water and Na^+ content during the luteal phase progesterone peak, although clearly this system is not involved during OC E + P or OC P administration.

We found that oral contraceptive pills containing estradiol led to a lower osmotic operating point for body fluid regulation, similar to that found during the luteal phase. These data suggest that estradiol has the primary effects on body fluid regulation during oral contraceptive administration and indicate that the progestins in oral contraceptives do not have a major effect on osmotic regulation of AVP and thirst. However, more research is needed to determine possible effects of elevations in endogenous progesterone on osmoregulation and the other components of body fluid regulation, such as fluid distribution and Na^+ regulation.

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In conduct of research where humans are the subjects, the investigators adhered to the policies regarding the protection of human subjects as prescribed by 45 CFR 46 and 32 CFR 219 (Protection of Human Subjects).

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